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KARYOTYPIC STUDIES IN SIX SPECIES OF APHIDS (HOMOPTERA : APHIDIDAE) FROM THE GARHWAL HIMALAYAS

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(Received 22 March 1980)

Somatic chromosome number and morphology in embryos of apterous viviparous females of *Amphicercidus* n. sp., *Cavariella* n. sp., *Macrosiphum rosae* (LINN.), *Macrosiphum rosaeformis* DAS, *Aiceona retipennis* DAVID, NARAYANAN & RAJASINGH and the leaf-gall forming *Epipemphigus imaicus* (CHOLODKOVSKY) belonging to Aphididae collected in and around Mussoorie, U. P. have been studied. It was found to be $2n = 8$ in *A. n.sp.*, $2n = 10$ in *C. n. sp.* and *M. rosae*, and $2n = 18$ in *M. rosaeformis*, *A. retipennis* and *E. imaicus*. Idiogram of each species has been prepared from morphometrical data of chromosomes and the possible mechanism of karyotypic evolution has been discussed.

(Key words: chromosome number, morphology, embryos, apterous viviparous females, aphids)

INTRODUCTION

Out of about 4000 species of aphids taxonomically described, cytological investigations have so far been carried out meagrely on some 390 odd species (ROBINSON & CHEN, 1969; KUZNETZOVA & SHAPOSHNIKOV, 1973; GUT, 1976; see MANNA, 1979). Further, of some 700 species hitherto morphologically described in India, (GHOSH, 1979), only 18 species are cytologically known (KHUDA-BUKHSH & DATTA, 1978; KHUDA-BUKHSH, 1979; DATTA & KHUDA-BUKHSH, 1980; KURL & MISRA, 1978, 1979; see MANNA, 1979). Of the six species under present report, two species, viz., *Macrosiphum rosae* and *M. rosaeformis* had been cytologically investigated earlier (KUZNETZOVA & SHAPOSHNIKOV, 1973; MISRA & KURL, 1979).

MATERIALS AND METHODS

Viviparous apterous females of six species of aphids, viz., *Amphicercidus* n. sp. (S.P. MAITY & S. CHAKRABARTI—personal communication), *Cavariella* n. sp. (same authors—p.c.), *Macrosi-*

phum rosae (LINN.), *Macrosiphum rosaeformis* DAS, *Aiceona retipennis*, DAVID, NARAYANAN & RAJASINGH, and the leaf-gall forming *Epipemphigus imaicus* (CHOLODKOVSKY), were collected from the host plants *Lonicera* sp. (Caprifoliaceae), *Salix babylonica* (Salicaceae), *Rosa* sp. (Rosaceae), *Rosa* sp. (Rosaceae), *Machilus* sp. (Lauraceae) and *Populus* sp. (Salicaceae) respectively in and around Mussoorie, U.P. and their embryos were subjected to a modified squash method described elsewhere (KHUDA-BUKHSH & DATTA, 1979) for studying their somatic chromosomes. According to the conventional method, idiogram based on the mean length values of chromosome set determined from 10 well-spread metaphase plates of each species was prepared.

RESULTS

The diploid numbers of 8 chromosomes in *Amphicercidus* n.sp. (Fig.1), 10 chromosomes each in *Cavariella* n. sp. (Fig. 2) and *M. rosae* (Fig. 3) and 18 chromosomes each in *M. rosaeformis* (Fig.4), *A. retipennis* (Fig.5) and *E. imaicus* (Fig.6) were determined from about 50 metaphase complements in each species. Analysis of the data of the mean length and the relative percentage length (RL) of individual chromosomes in all the

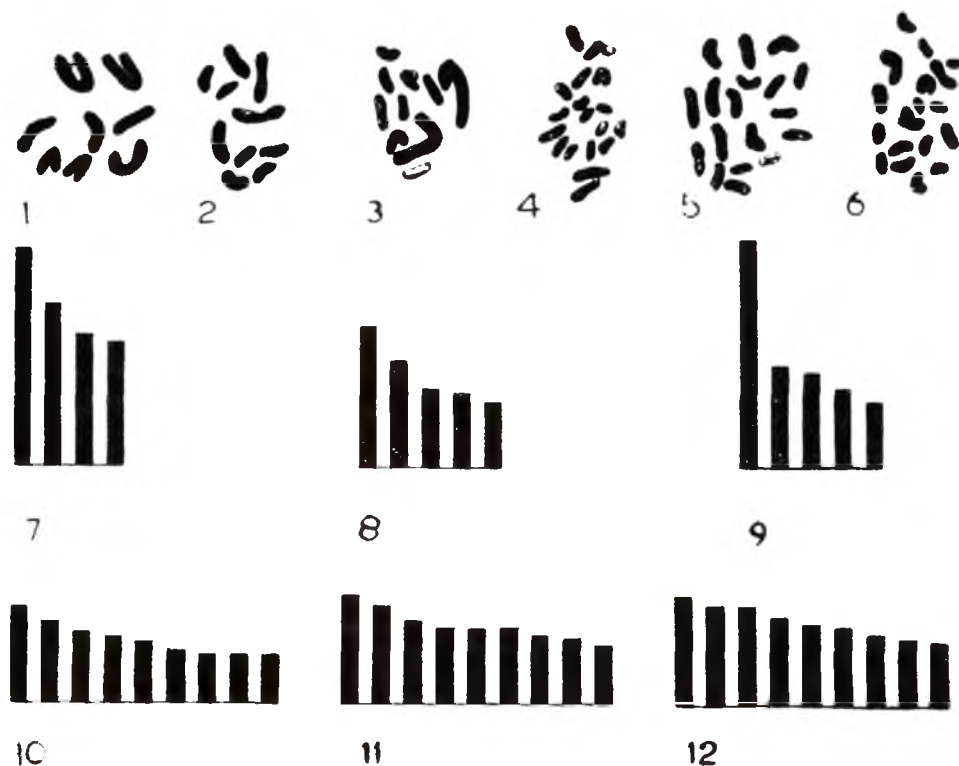
six species (Table 1) as well as their idiograms (Figs. 7-12) would reveal that the chromosomes were gradually seriated in all but *M. rosae* (Fig. 9) which had the 1st pair of chromosomes appreciably longer (by 3.07 micra) than the 2nd pair. In *Amphicercidus* n.sp., the difference between 1st and 2nd pairs was also to some extent palpable, the difference being 1.34 micra (Table 1), although it was not as conspicuous as in *M. rosae*. The gradual seriation was more notable in *M. rosaeformis* (Fig. 10), *A. retipennis* (Fig. 11) and *E. imaicus* (Fig. 12) and some chromosomes, e.g. Nos. 7-9 in *M. rosaeformis* Nos. 5-6 in *A. retipennis* and Nos. 2-3 in *E. imaicus*, seemed to be of equal lengths (Table 1).

The absence of primary constriction in chromosomes and the sheet-like parallel

poleward movement of daughter chromosomes in all the six species indicated their holokinetic nature of chromosomes.

DISCUSSION

Among 4 species of the genus *Cavariella* cytologically investigated (see MANNA, 1979), 3 viz., *C. (Hydronaphis) cerauthi* (MAKINO, 1956), *C. oenanthi* (KUZNETSOVA & SHAPOSHNIKOV, 1973) and *C. pastinacae* (GUT, 1976) had $2n=8$ chromosomes while *Cavariella* sp. reported by ROBINSON & CHEN (1969) had $2n=10$ chromosomes. In the present investigation, *Cavariella* n.sp. not only possessed $2n=10$ chromosomes, but also the chromosomes were morphologically more alike to that of *Cavariella* sp. (ROBINSON & CHEN, 1969) except for some difference in



Metaphase complements in embryos of *Amphicercidus* n.sp. (Fig. 1), *Cavariella* n.sp. (Fig. 2), *M. rosae* (Fig. 3), *M. rosaeformis* (Fig. 4), *A. retipennis* (Fig. 5) and *E. imaicus* (Fig. 6). Figs. 7-12. Idiograms of the above mentioned species respectively.

TABLE 1. The mean length and relative percentage length (RL) values of chromosome sets in six species of aphids.

Sl. No. of Chromosomes	Amphid. n. sp.		Cavari n. sp.		Mac. rosae		M. rosaeformis		A. retipennis		E. imaicus	
	Mean length (μ)	RL	Mean length (μ)	RL	Mean length (μ)	RL	Mean length (μ)	RL	Mean length (μ)	RL	Mean length (μ)	RL
1	5.38	34.1	3.46	30.2	5.57	39.9	2.38	17.1	2.61	15.0	2.61	17.4
2	4.04	25.5	2.61	22.8	2.50	18.0	2.00	14.4	2.38	13.7	2.00	13.3
3	3.27	20.7	1.92	16.8	2.37	16.6	1.69	12.2	2.00	11.5	2.00	13.3
4	3.07	19.4	1.84	16.1	1.92	13.7	1.61	11.6	1.92	11.0	1.69	11.3
5	1.61	14.1	1.61	11.5	1.46	10.5	1.84	10.6	1.53	10.3
6	1.31	9.4	1.84	10.6	1.46	9.7
7	1.15	8.2	1.69	9.8	1.31	8.7
8	1.15	8.2	1.61	9.3	1.23	8.2
9	1.15	8.2	1.46	8.4	1.15	7.7

relative sizes between the 2nd and 3rd chromosomes. In the present species it was more prominent than in the one described by ROBINSON & CHEN (1969).

So far as the present author is aware, cytological investigations in any congeneric species of *Amphicercidus*, *Aiceona* and *Epipemphigus* had not been carried out earlier for which karyotypic comparison among congeneric species in these genera was not possible. Taxonomically, *Aiceona* and *Epipemphigus* belong to two different sub-families, namely, Anoecinae and Pemphiginae respectively although both the species possess the same diploid number of 18 chromosomes. Though some difference in the absolute genome lengths and the relative percentage lengths of individual chromosomes was observed between these two species (Table 1), no obvious cytological characterization of karyotypes in these two sub-families was possible.

Altogether 18 species including an unidentified one belonging to *Macrosiphum* (see MANNA, 1979; KURL & MISRA, 1979) have so far been cytologically known of which 11 species have $2n = 10$, 3 species have $2n = 14$, 2 species have $2n = 18$ and 1 species each has $2n = 8$ and 12 chromosomes indicating thereby the diploid modal number likely to be $2n = 10$ in this genus. The chromosome numbers of 10 in *M. rosae* and 18 in *M. rosaeformis* reported earlier by KUZNETZOVA & SHAPOSHNIKOV (1973) and KURL & MISRA (1979) respectively are in agreement with the present findings. Though the diploid numbers in these two congeneric species were strikingly different, the absolute genome lengths—13.97 micra in *M. rosae* and 13.90 in *M. rosaeformis*—were very close to each other. This would suggest that fission/fusions of chromosomes were involved in the evolution of karyotypes in these two species as is also true of many other species of aphids (ROBINSON & CHEN,

1969; KUZNETZOVA & SHAPOSHNIKOV, 1973; KHUDA-BUKHSH & DATTA, 1978; KHUDA-BUKHSH, 1979).

Opinions differ among cytologists as to the possible modal number in the aphids in general. While SUN & ROBINSON (1966) suggested $2n = 8$ as the possible primitive number, CHEN (1968) was inclined to believe $2n = 4$ to be the likely modal number in aphids. Recently, MANNA (1979) compiled the available data on the chromosome numbers of aphids and found that while only 3 species had $2n = 4$ chromosomes, 75 species belonging to 32 genera had $2n = 8$ chromosomes against 135 species distributed over 50 genera having $2n = 12$ chromosomes. He, therefore, suggested most likely $2n = 12$ chromosomes to be the modal number in aphids from which structural rearrangements in form of fusion/fission played the key role in the evolution of aphid karyotypes.

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CYTOTAXONOMY OF THE GENUS *TOXOPTERA* (HOMOPTERA : APHIDIDAE)

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(Received 15 April 1980)

Three species, viz., *T. aurantii*, *T. citricidus* and *T. odinae* have been worked out cytologically (somatic), using air-dry Giemsa technique. In all the species the chromosome numbers were found to be $2n = 8$. The chromosomal morphology and their metrical data are recorded and the cytotaxonomy of the species has been discussed.

(Key words: chromosomes, cytotaxonomy of aphids, *Toxoptera*)

INTRODUCTION

The members of genus *Toxoptera* of the tribe Aphidini, are well known pests of fruit trees (GHOSH, 1975 a,b). The genus includes 39 known species (EASTOP & HILLERIS LAMABERS, 1976) widely distributed throughout the tropical, sub-tropical and temperate regions of the world. In India this genus is represented by three species (GHOSH, 1975 a,b) mainly confined to eastern and southeastern parts of the country. Out of 39 species known taxonomically only one species, *Toxoptera aurantiae* (BOYER DE FONSCOLOMBE) is so far known cytologically (KURL & MISRA, 1980 b). It was PAGLIAI (1961) who for the first time studied its mitosis and meiosis in Italy and reported its diploid chromosome number ($2n = 8$).

In the present communication the only three species available in India, viz., *Toxoptera aurantii* (BOYER DE FONSCOLOMBE), *T. citricidus* (KIRKALDY) and *T. odinae* (VAN DE GOOT) have been investigated cytologically with a view to understand the relationship between the congeneric species.

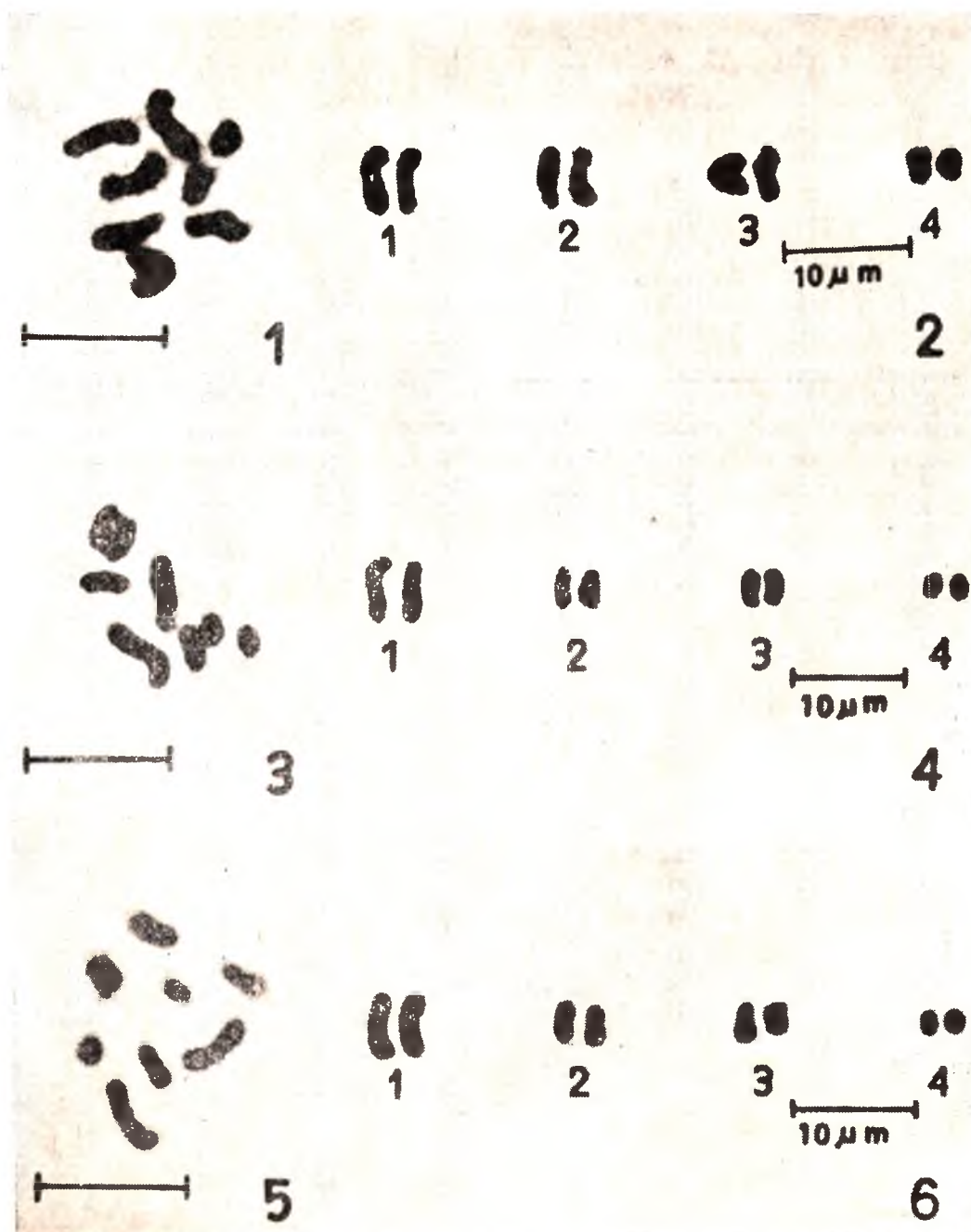
MATERIALS AND METHODS

The aphids were collected from Assam and Meghalaya states from their host plants mentioned

below: *Toxoptera aurantii* (*Evodia fraxinifolia*, *Eurya* sp., *Symplocos* sp., *Symplocos paniculata*); *T. citricidus* (*Citrus acida*, *Rhus khasiana*); *T. odinae* (*Viburnum foetidum*, unidentified shrub). Embryos of the aphids for all samples were dissected out from apterous viviparous females and permanent cytological preparations were made following the air-dry method (KURL & NARANG, 1978) using Giemsa stain (10% in phosphate buffer at pH 6.8). For metrical data well spread metaphase plates were selected and the actual lengths of chromosomes were measured. Later on the photomicrographs were taken. Projections of the photomicrographs were then traced on a white paper with a sharp pencil for determination of the relative percentage length of the each chromosome. The mean percentage for all the chromosomes of each species were finally expressed in relative percentage length. Assuming the chromosomes of approximately same lengths to be homologous, the pairing of homologous chromosomes were made and then the karyotypes were constructed (Figs. 2.4 & 6).

RESULTS

The diploid chromosome numbers in all the species, viz., *T. aurantii*, *T. citricidus* and *T. odinae* were found to be 8 (Figs. 1,3 & 5), which were hitherto unknown. In *T. aurantii*, out of 46 well-spread metaphase plates examined, more than 70% contained $2n = 8$ while rest of the plates had aneuploid and polyploid numbers. In *T. citricidus*, 95% metaphase plates had $2n = 8$ chromosomes while the remaining had $2n = 7$



Figs. 1 to 6. Somatic chromosomes of aphids. Figs. 1 & 2. *T. aurantii*: 1, metaphase; 2, karyotype. Figs. 3 & 4; *T. citricidus*. 3, metaphase; 4, karyotype. Figs. 5 & 6: *T. odinae*. 5, metaphase; 6, karyotype.

chromosomes. In *T. odinae* after examining 38 cells, the diploid chromosome number has been established $2n = 8$.

Since no sexual form of the present species has been reported from India and the author did not get any sexual form, the meiosis could not be studied. However, detailed observations on mitotic cell division in the three species were made.

In all the species no heterochromatin body was observed during interphase. In prophase irregularly twisted threads appeared. In prometaphase the chromosomes were long and took light stain. At metaphase the chromosomes were condensed, short, rod shaped and took dark stain (Figs. 1, 3 & 5). No anaphase and telophase have been observed. In all the chromosomes no primary or secondary constriction could be observed.

The actual lengths in microns and the relative percentage lengths of the somatic metaphase chromosomes are given in Table 1. At prometaphase the chromosomes are relatively long. The actual lengths of chromosomes at pro-metaphase in *T. aurantii* range from 4.29 to 11.44 microns; in *T. citricidus* from 3.48 to 10.97 microns and in *T. odinae* 2.86 to 10.07 microns. In *T. aurantii* the actual lengths of the diploid number of chromosomes at metaphase range from 7.98 to 2.97 microns, in *T. citricidus* from 7.15 to 2.86 microns while in *T. odinae* from 8.72 to 2.71 microns.

In all the species the homologous pairs (assuming chromosomes of similar length to be homologous) were selected and the karyotypes were constructed (Figs. 2, 4 & 6). The relative percentage length of each chromosome and the total complement length (TCL) for each species are given in Table 1. In all the species there are one long pair, two

medium-length pairs and one short pair of chromosomes. In *T. odinae*, the longest chromosome is approximately 3 times bigger than the shortest while in *T. aurantii* and *T. citricidus*, it is 2.5 times bigger than the shortest.

DISCUSSION

The only species (*T. aurantiae*) cytologically known, of the genus *Toxoptera* of sub-tribe Aphidina shows the chromosome number $2n=8$ (PAGLIAI, 1961) and in the present study all the three species investigated show $2n = 8$ chromosomes. The other genus, *Aphis* of the sub-tribe Aphidina also shows the chromosome numbers $2n=8$ in 31 species worked out so far except one species *A. oenotherae* in which the number is $2n = 10$ (KURL & MISRA, 1980b). Moreover, if we consider the chromosome numbers of the sub-family Aphidinae, here majority of the species have $2n=8$. It has been established that the modal number of this subfamily is 8 (GUT, 1976) but the chromosomes range from $2n=4$ to $2n=40$. For the two genera (*vide supra*), many species that have been examined, show remarkable constancy in their chromosome numbers.

It is generally believed that the chromosomes of aphids either have a diffuse centromere activity or are holocentric (SCHRAEDER, 1935; HUGHES-SCHRADER, 1948; HUGHES-SCHRADER & SCHRADER, 1961; WHITE, 1973; KURL, 1978; KURL & MISRA, 1980a). In the present species no chromosome with localized centromere was found either in prophase, prometaphase or metaphase, confirming the holocentric nature of chromosomes.

Since, aphid chromosomes lack distinct morphological features, HARPER & MACDONALD (1966) and OLIVE (1967) pointed out that the comparisons of karyotypes are

TABLE 1. Actual lengths in microns and relative percentage lengths of somatic metaphase chromosomes of three species of *Toxoptera*.

Species	No. of complements measured	T. C. L.	Mean chromosome length							
			Chromosome number							
			1	2	3	4	5	6	7	8
<i>T. aurantii</i>	16	41.18 μ ± 3.04	7.98 ± 0.75	7.98 ± 0.75	5.48 ± 0.29	5.48 ± 0.29	4.16 ± 0.28	4.16 ± 0.28	2.97 ± 0.34	2.97 ± 0.34
	14	"	16.55 ± 0.90	15.48 ± 0.39	14.70 ± 0.55	13.50 ± 0.53	13.28 ± 0.59	12.92 ± 0.78	8.85 ± 0.53	8.29 ± 0.45
<i>T. citricidus</i>	13	38.12 μ ± 0.46	7.15 ± 0.00	7.15 ± 0.00	4.76 ± 0.23	4.76 ± 0.23	4.29 ± 0.00	4.29 ± 0.00	2.86 ± 0.00	2.86 ± 0.00
	12	"	18.70 ± 0.34	17.12 ± 1.20	12.36 ± 0.46	12.14 ± 0.23	11.66 ± 0.71	10.33 ± 0.37	9.15 ± 0.35	8.47 ± 0.14
<i>T. odinae</i>	15	41.72 μ ± 2.09	8.72 ± 0.56	8.72 ± 0.56	5.29 ± 0.28	5.29 ± 0.28	4.14 ± 0.26	4.14 ± 0.26	2.71 ± 0.14	2.71 ± 0.14
	12	"	20.07 ± 0.55	19.42 ± 0.09	11.95 ± 0.89	11.39 ± 0.51	10.63 ± 0.31	10.15 ± 0.14	8.21 ± 0.59	8.12 ± 0.51

not usually helpful for differentiation between congeneric species. This handicap can be got over by the measurements of relative lengths and volumes, of the somatic chromosomes, thus making the cytotaxonomy fruitful. The TCL and the mean chromosome lengths of the three species examined are almost identical (Table 1) but even then, they are separate species. There is yet no cytological explanation for it. Secondly, several authors published the relative lengths and idiograms of some cosmopolitan species but no constancy in their results has been observed. Apparently this subject is considerably more complicated than the available literature suggests (GUT, 1976).

There is yet no agreement on the modal number of chromosomes in aphids. Modal numbers $2n=8$ chromosomes (SUN & ROBINSON, 1966), $2n=4$ chromosomes (CHEN, 1968) and $2n=6$ chromosomes (SHINJI, 1931) have been suggested but there is still disagreement about, whether high or low numbers are most primitive.

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EFFECTS OF FEEDING SENESCENT LEAF OF *CALOTROPIS GIGANTEA* ON FOOD UTILISATION IN THE MONARCH BUTTERFLY *DANAUS CHRYSIPPUS*

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Energy and protein contents of senescent *Calotropis gigantea* leaf are less in comparison to the normal leaf. As many as 80% I instar or 47% final instar *Danaus chrysippus* larvae fed on senescent leaf suffered mortality. Besides they extended the larval duration from 9 days in those feeding on normal leaf to 29 days. Consequent to the extension of larval duration, food energy consumed by the senescent leaf fed larvae was more (5079 gcal) than that (2751 gcal) fed on normal leaf. However, their efficiencies of assimilation (36%) and net conversion (13%) were less than those fed on normal leaf. Consumption, assimilation and net conversion of leaf protein by the larvae fed on senescent leaf were significantly less than those fed on normal leaf.

(Key words: *Danaus chrysippus*, senescent leaf, mortality, food, utilisation)

INTRODUCTION

Green leaf is an excellent source of most nutrients for majority of insects (FRAENKEL, 1953, 1959). As the leaf becomes senescent, its composition significantly differs from that of the normal leaf especially in protein and chlorophyll content (see DAS, 1968). In natural habitat, *Danaus chrysippus* mostly feeds on the normal green leaf of *Calotropis gigantea* and at the time of defoliation of the plant it switches over to senescent leaf. Despite a good number of publications on food utilisation in herbivorous insects, information on the effect of feeding senescent leaf on food utilisation in insect is lacking. The present paper deals with utilisation of senescent *C. gigantea* leaf by larvae of *D. chrysippus*.

MATERIAL AND METHODS

Freshly hatched/moulted *Danaus chrysippus* larvae of II, III, IV and V instars were collected from *Calotropis gigantea* plant in the field near Madurai Kamaraj University. They were weighed in a single pan balance (accuracy 0.01 mg) and reared individually in plastic terraria (capacity 400 ml).

Two series of experiments were conducted. In the first series, the larvae were offered weighed quantities of fresh normal *C. gigantea* leaf and for those in the second series yellow senescent leaf was offered. Both sets of experiments were run simultaneously at $29 \pm 1.5^\circ\text{C}$, 80 ± 10 r.h. under a source of even illumination of 10 L: 14 D a day. Since the larvae fed on senescent leaf died (80% of I instar and 47% of final instar) just after moulting, it was not possible to estimate food utilization with the same larva from hatching to terminations of feeding period. When a test larva feeding on senescent leaf succumbed, another larva of same stage was recruited from the stock. To estimate food consumption, standard gravimetric method WALDBAUER (1968) was followed. Samples of food, feces, test larvae were dried to weight constant at 90°C . The scheme of energy balance followed was the IBP formula of PETRUSEWICZ & MACFADYEN (1970) usually represented as $C = P + R + F + U$, where C is the food consumed, P the growth, R the energy loss due to metabolism, F the feces and U the nitrogenous wastes. Quantitative estimation of consumption, assimilation, conversion and metabolism were made following the method described by MATHAVAN & BHASKARAN (1975). Calorific content was determined in a Parr 1411 semi micro bomb calorimeter.

Biochemical analyses of the test materials:

Chlorophyll content of the normal and senescent leaf was determined following ARNON (1949). Carbohydrate content of the chlorophyll-free normal and

senescent leaves was estimated following the method of DUBOIS *et al.* (1956). Protein content of chlorophyll-free leaves, test larvae and their feces was estimated following LOWRY *et al.*, (1951). Modified method of RAYMONT *et al.* (1958) was adopted for lipid estimation. As recommended by PAVLYUTIN (1970), ash content of the leaves was determined.

RESULTS

Normal and yellow senescent leaves of *C. gigantea* varied in their caloric density and chemical composition (Table 1). The senescent leaf contained 49.6 mg protein/g dry weight as against 185.2 or 101.4 mg/g in the normal leaf or its latex. However, the carbohydrate and lipid contents of the latex were more than those of normal and senescent leaves. Cellulose, lignin, crude fibres, phenols and nucleic acids represent the minor constituents of the leaves. The normal leaf was rich in energy content (4560 gcal/g) compared to the senescent leaf (4051 gcal/g).

Larvae fed on normal leaf required a feeding period of 9 days to complete their development; those feeding on senescent leaf required as many as 29 days. Pupal duration was around 6 days in both the series.

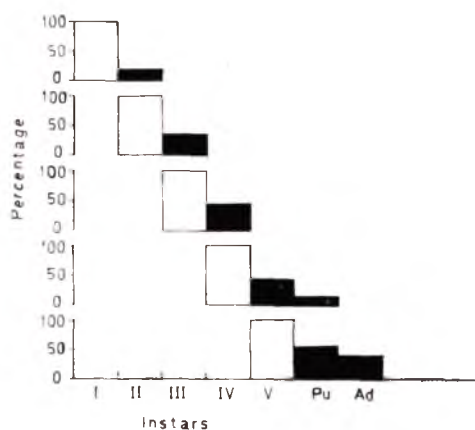


Fig. 1 Survival (%) of *D. chrysippus* larvae fed on senescent *C. gigantea*. Freshly moulted larvae of the different instars were tasted for survival.

Final live weight of the larvae at the termination of larval stage in the senescent leaf fed series was 350 mg as against 645 mg in that fed on normal leaf. About 16% of the final instar larvae in the senescent leaf fed series became half pupa and subsequently succumbed. Only 20, 33, 45, 48 and 53% of I, II, III, IV and V instar larvae in the senescent leaf fed series survived and managed to pass on to the subsequent life stage (s)

TABLE 1. Differences in the chemical composition of normal and senescent *C. gigantea* leaf and the latex. Values on chlorophyll, carbohydrates, fat and protein are expressed in terms of mg/g dry weight and those of energy content in terms of gcal/g dry matter.

Sample	Water %	Chlorophyll	Carbohydrates	Protein	Lipid	Ash	Energy
Normal leaf	84	6.3	363.6	185.2	265.4	39.7	4560
	± 6.8	± 1.12	± 23.12	± 3.14	± 16.1	± 1.32	± 67.3
Latex	83.7	0	463.4	101.4	358.7		5485
	± 9.24		± 38.23	± 17.62	± 42.51		± 19.38
Senescent leaf	88	1.2	296.7	49.6	230.1	28.4	4051
	± 7.5	± 0.23	± 18.96	± 4.5	± 10.55	± 2.2	± 123.3

TABLE 2. Energy budget of larva of the monarch butterfly *Danaus chrysippus* fed *ad libitum* on normal (N) and senescent (S) *Calotropis gigantea* leaf. Energetics data for such of those final instar larvae (in the senescent leaf fed series) which became half pupa are also provided. All values ($\bar{x} \pm S.D.$) are expressed in cal as a function of instar.

Instar	Instar duration (days)	Consumption	Defecation	Conversion	Assimilation efficiency (%)	Conversion efficiency (K_2) (%)
I	N 1.0 \pm 0.0	10.5 \pm 2.28	1.1 \pm 0.36	0.53 \pm 00.05	89.9 \pm 22.56	5.61 \pm 0.56
	S 4.0 \pm 1.0	17.0 \pm 2.98	4.4 \pm 0.85	0.52 \pm 0.07	74.7 \pm 2.95	4.0 \pm 0.71
II	N 1.5 \pm 0.5	44.0 \pm 6.38	8.01 \pm 1.02	6.1 \pm 0.51	81.9 \pm 13.32	16.8 \pm 1.52
	S 3.0 \pm 0.0	43.8 \pm 3.19	14.4 \pm 0.79	3.4 \pm 0.24	67.2 \pm 2.12	11.6 \pm 0.38
III	N 2.0 \pm 0.0	106.3 \pm 9.12	31.7 \pm 4.83	13.6 \pm 1.21	70.1 \pm 9.67	18.2 \pm 1.08
	S 5.0 \pm 1.0	383.1 \pm 68.34	212.8 \pm 36.65	20.6 \pm 2.07	44.5 \pm 7.31	12.1 \pm 0.83
IV	N 2.0 \pm 0.5	371.6 \pm 71.59	115.8 \pm 28.4	66.9 \pm 7.91	68.9 \pm 25.14	26.2 \pm 2.89
	S 6.0 \pm 1.0	976.5 \pm 81.91	558.1 \pm 84.31	55.5 \pm 2.83	42.8 \pm 3.31	13.3 \pm 0.87
V	N 3.0 \pm 0.5	2219.1 \pm 604.22	940.1 \pm 131.35	527.3 \pm 33.79	57.6 \pm 17.19	41.2 \pm 2.98
	S 11.0 \pm 1.8	3658.8 \pm 268.51	2448.1 \pm 318.24	165.0 \pm 23.95	33.1 \pm 5.92	13.6 \pm 1.03
Half pupa	10.0 \pm 1.4	3715.3 \pm 698.35	1948.9 \pm 376.75	126.5 \pm 19.75	47.5 \pm 11.69	7.2 \pm 1.22

(Fig.1). Fig 2 shows the difference in size of the pupa in normal and senescent leaf fed series as well as the half pupa.

Instar wise energy budget of *D. chrysippus* larvae fed *ad libitum* on normal or senescent leaf is provided in Table 2. With advancing life stages food energy consumed, defecated and converted increased. As the larvae grew from I to V instar, assimilation efficiency decreased from 90 to 58% in the normal leaf fed series and 75 to 33% in that fed on senescent leaf (Fig.3). Correspondingly, net conversion efficiency of the larva increased from 4 to 14% in the senescent leaf fed series and 6 to 41% in the normal leaf fed series (Fig.3). Corresponding to the increase in food consumption with advancing age, quantity of protein consumed, defecated and converted increased (Table 3). The final instar larvae feeding on normal leaf consumed 90 mg of leaf protein and converted about 12% into its body protein. In the senescent leaf fed

series, protein consumption and conversion during the final instar amounted to 45 and 3 mg, respectively.

During the entire feeding period, a larva fed on normal leaf consumed, defecated and converted 2752, 1097 and 611 gcal respectively. Corresponding values for a larva in the senescent leaf fed series were 5079, 3238 and 245 gcal respectively (Table 4). Statistically significant differences between normal and senescent leaf fed larvae were observed in the amount of protein consumed, defecated and converted (Table 4). Larvae fed on normal leaf consumed 112 mg, defecated 31 mg and converted 13 mg leaf protein. Due to the remarkable decrease in the protein content of the senescent leaf, protein consumption, defecation and conversion by the larva reduced to 62, 24 and 5 mg respectively. Those larvae which became half pupa consumed 51 mg leaf protein and converted only 3.2 mg of it.

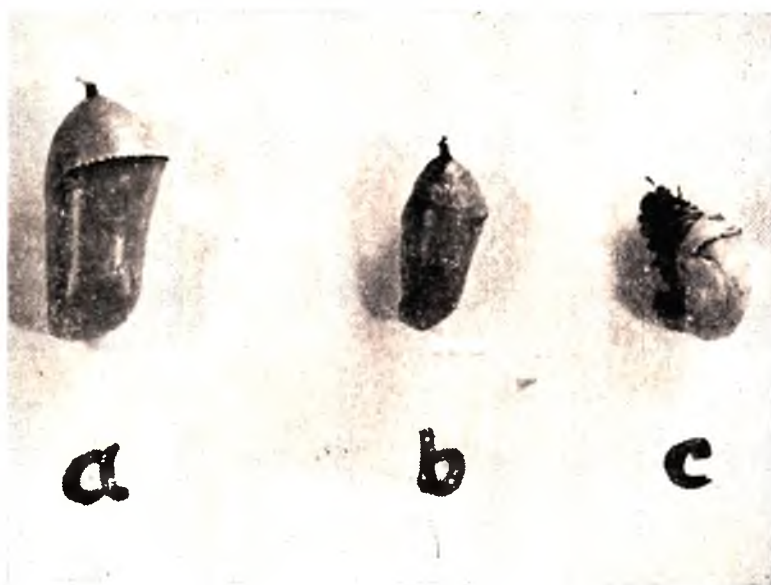


Fig. 2 a. *Danaus chrysippus* pupa in the normal leaf fed series. b. *D. chrysippus* pupa in the senescent leaf fed series. c. Half pupa in the senescent leaf fed series.

DISCUSSION

Feeding senescent *C. gigantea* leaf resulted in a number of adverse effects on *D. chrysippus*. They are: 1. mortality, 2. extension of instar duration, 3. decrease in food utilisation efficiencies and 4. decrease in final body weight and production of "mini" pupa and adult. JOHNSON (1969) emphasised the importance of size of lepidopterous adults which affects dispersal and reproductive success. These negative effects can be attributed to the low energy and protein contents of the senescent leaf. One of the important strategies in insect development is to postpone metamorphosis until all the necessary materials are accumulated to become a sexy adult (WILLIAMS, 1970). Availability of nitrogen for the synthesis of new cuticle is one of the factors regulating moulting (GILBERT, 1964). Obviously, *D. chrysippus* larva seems to have extended the instar duration so as to accumulate the required amount of energy.

For instance, 47% of the larvae feeding senescent leaf failed to complete pupation and died, as they converted less than the critical levels of protein and energy requirement. In the senescent leaf fed series, the larvae which became half pupa converted 151 gcal of energy and 3.2 mg of protein in the entire feeding period. Hence the critical levels of conversion for successful pupation and emergence are above 151 gcal for energy and 3.2 mg for protein.

Working on the effect of nitrogen content of the feed on the mortality of the grasshopper *Melanoplus mexicanus mexicanus*, SMITH & NORTHCOTT (1951) found that hoppers feeding on wheat with 6% nitrogen content suffered only 52% mortality as against 100% mortality of those reared on wheat containing 3% nitrogen. They also observed extension of hopper period fed on wheat containing less nitrogen. These findings of SMITH & NORTHCOTT (1951)

TABLE 3. Protein budget of larva of the monarch butterfly *Danaus chrysippus* fed *ad libitum* normal (N) and senescent (S) *Calotropis gigantea* leaf. Data on protein utilisation for such of those final instar larvae (in the senescent leaf fed series) which became half pupa are also provided. All values (\bar{x} — SD) are expressed in mg as a function of instar.

Instar	Consumption	Defecation	Conversion
I	N 0.43 \pm 0.99	0.03 \pm 0.01	0.011 \pm 0.001
	S 0.21 \pm 0.04	0.03 \pm 0.01	0.006 \pm 0.001
II	N 1.79 \pm 0.25	0.23 \pm 0.05	0.13 \pm 0.01
	S 0.54 \pm 0.10	0.11 \pm 0.04	0.04 \pm 0.01
III	N 4.31 \pm 0.37	0.90 \pm 0.14	0.22 \pm 0.04
	S 4.69 \pm 0.84	1.59 \pm 0.24	0.34 \pm 0.08
IV	N 15.1 \pm 2.91	3.29 \pm 0.81	1.41 \pm 0.17
	S 11.9 \pm 1.01	4.16 \pm 0.85	1.01 \pm 0.18
V	N 90.12 \pm 24.58	26.69 \pm 3.73	11.14 \pm 0.71
	S 44.82 \pm 13.31	18.27 \pm 2.51	3.46 \pm 0.98
Half pupa	45.5 \pm 8.61	14.5 \pm 1.53	2.70 \pm 0.87

TABLE 4. Bioenergetics of the *D. chrysippus* larva fed *ad libitum* on normal and senescent *C. gigantea* leaf. Values are expressed in kcal (energy) and mg (protein), and represent the average performance of not less than 5 larvae.

Parameter	Normal leaf			Senescent leaf		
	Energy	Protein	Normal pupation	Energy	Half pupation	Protein
Consumption	2752A \pm 693.6	112* \pm 28.2	5079A \pm 693.6	4158 \pm 772.5		51 \pm 9.6
Defecation	1097B \pm 166.3	31** \pm 4.7	3238B \pm 440.8	2180 \pm 414.9		16 \pm 1.8
Assimilation	1655 \pm 246.2	81 \pm 22.7	1841 \pm 519.9	1978 \pm 345.25		35 \pm 7.87
Conversion	611C \pm 42.8	13*** \pm 0.9	245C \pm 29.2	151 \pm 22.13		32 \pm 0.96
Metabolism	1044 \pm 202.9	68 \pm 21.9	1596 \pm 227.1	1827 \pm 323.1		31 \pm 6.8
Assimilation efficiency (%)	60.0	72.2	36.3	47.6		68
Conversion efficiency (K ₂)	36.9	16.0	13.3	7.6		9.3

Energy
A P < 0.01
B P < 0.001
C P < 0.001

Protein
* P < 0.05
** P < 0.05
*** P < 0.001

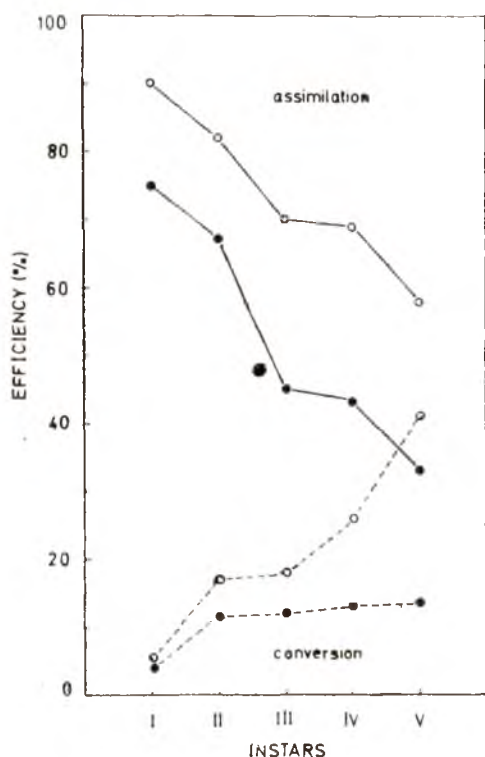


Fig. 3 Efficiencies of assimilation (continuous line) and net conversion (discontinuous line) of *D. chrysippus* larva fed *ad libitum* on normal (open circle) and senescent (filled circle) *C. gigantea*.

support the conclusion that low levels of energy and nitrogen contents in the senescent leaf are responsible for the observed adverse effects on *D. chrysippus*. MATHAVAN & BHASKARAN (1975) concluded that nutritional inadequacy and less amount of latex in *Asclepias curssavica* compared to those in *C. gigantea* is responsible for the decrease in food consumption of *D. chrysippus* larva. Surprisingly, despite less latex content in the senescent *C. gigantea* leaf, *D. chrysippus* larva consumed more energy from the senescent leaf than that from the normal leaf. This is likely to be due to the prolongation of larval duration in the senescent leaf fed series and the latex content of the leaf may not have any correlation with the quantity of food consumed.

The decrease in the assimilation of the larvae feeding senescent leaf may be due to the high percentage (39%) of less energy containing components like cellulose, crude fibers, lignin in the senescent leaf. BAILEY (1976) attributed the low assimilation efficiency of larva of the armyworm *Mamestra configurata* to selective feeding on parts of the leaf containing more crude fibers. Larvae of *Achea junta* also exhibited low efficiency due to selective feeding on less energetic components (MUTHUKRISHNAN, 1980).

In the senescent leaf fed series the larvae exhibited very low conversion efficiency (13.3%). Protein content of the feed is likely to have significant influence on the conversion efficiency. DESHIMARU & SHIGENO (1972) found that conversion efficiency of the prawn *Penaeus japonicus* increased with increasing protein levels in the feed. Obviously, the low levels of protein in the senescent leaf is responsible for the low conversion efficiency of *D. chrysippus*.

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DIETARY WATER BUDGET IN *MYLABRIS INDICA* (COLEOPTERA : MELOIDAE)

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The dietary water intake and loss via defecation, absorption, retention and transpiration in *Mylabris indica* fed on *Hibiscus rosasinensis* were estimated. Rates of dietary water intake, absorption, retention and transpiration were inversely proportional to the weight of the beetles. The very poor dietary water retention capacity and significantly greater transpiration of dietary water are suggested to be an adaptive mechanism for the active flight activity in *M. indica*.

(Key words: *Mylabris indica*, *Hibiscus rosasinensis*, dietary water, transpiration, flight activity)

INTRODUCTION

Mylabris indica is a polyphagous pest and feeds exclusively on the flowers of *Hibiscus rosasinensis*, *H. esculantus*, *Dolichos lablab*, *D. tribolus*, *Tephrosia purpurea*, *Cucurbita peo*, *Trichosanthus angunia*, groundnut, cotton and red gram (SHUKLA & UPADHYAYA, 1973; VASANTHARAJ DAVID & KUMARASWAMI, 1978). In a previous publication, the damage caused by *M. indica* to the flower of *H. rosasinensis* has been reported (CHOCKALINGAM & MANOHARAN, 1979). The present paper deals with the dietary water budget of *M. indica* fed on *H. rosasinensis*.

MATERIALS AND METHODS

Mylabris indica, collected from the local gardens, were divided into three groups based on their hind wing size and body weight. All weights were taken in a monopan balance sensitive to 0.01 mg. Beetles were maintained in separate containers (6"×4"×4") for 30 days in an incubator at an average temperature of $29 \pm 1^\circ\text{C}$ and RH $74 \pm 5\%$.

Since the beetles were found to feed heavily during the dusk and dawn, weighed fresh flowers of *Hibiscus rosasinensis* were offered daily at 5 PM. It is likely that certain amount of water could have evaporated from the flowers offered before they were ingested. A standard estimation revealed that the

transpiratory water loss from the flower prior to consumption amounted to 0.9% of intake of water.

The intake of dietary water, loss of water via defecation, absorption and retention of water by *M. indica* were calculated following the method employed by PANDIAN *et al.* (1978).

Water content of fresh flower of *Hibiscus rosasinensis* was 80%. Using this standard value of water in the flower and the total amount of flower consumed by the insect in 30 days, the quantity of water intake was determined.

Freshly laid fecal pellets were collected periodically once in four hours at different times in all the days of experiment, weighed and dried at 95°C to a constant weight. Using the values of mean water content in freshly laid fecal pellets and total dry weight of feces, the total water loss via defecation was estimated.

To estimate the retention of ingested water, beetles of various size groups were selected and weighed individually before feeding and then they were dried at 95°C to weight constancy. The difference in the weight was taken as an initial water content of the beetles selected for experiment. Beetles were fed continuously for 30 days, weighed and then dried to weight constancy to obtain the value of final water content in the experimental beetles. The amount of water retained by a beetle in 30 days period was estimated by subtracting the initial water content from the final water content.

Absorption and retention efficiency of water were calculated relating the quantity of absorbed and retained water.

RESULTS AND DISCUSSION

Data on the dietary water intake, absorption, retention, the loss *via* feces and transpiration for 30 days period are given in Table 1. The intake ranged from 2119 to 3948 mg for males and 2368 to 3923 mg for females. The water intake averaged to 3189 mg in males and 3349 in females for 30 days. In both males and females, dietary water intake was found to be appreciably increased with increased consumption of food.

Loss of water *via* feces averaged to 85 mg in males and 84 mg in females. Water absorbed from food was 3104 mg in males and 3265 mg in females. In both the sexes, the amount of water absorbed was found to be directly proportional to the quantity of food consumed. A notable variation observed was the amount of water retained between sexes; females retain more water (19 mg) than males (14 mg).

Water transpired ranged from 3090 to 3246 mg and it increased with the increase in the size of the beetles.

Rates of dietary water intake, absorption, retention and transpiration and efficiency of absorption and retention are given in Table 2. The mean rates of dietary water intake, absorption, retention and transpiration did not vary much between male and female. However, body weight influenced the rate of dietary water intake, absorption, retention and transpiration significantly. The smallest beetle (42 ± 2 mg) showed 2 1/2 times higher rates of water intake, absorption, retention and transpiration than the largest beetles (220 ± 3 mg).

Rate of water retention in smallest beetle was five times greater than that recorded for largest beetle. Water reserves in insects depend on size and metabolic water

produced from fat oxidation (BURSELL, 1970). Small insects are better assimilators than large insects (WIGGLESWORTH, 1965). Food intake rate in *M. indica* is greater in smallest beetles than in the largest beetles (CHOCKALINGAM & MANOHARAN, 1980). Corresponding with the greater rate of food intake, the dietary water intake rate is also greater in smallest beetles. The greater rate of water retention in small *M. indica* may be correlated with its greater rate of food ingestion.

The dietary water retention efficiency in *M. indica* was very poor, ranging from 0.50 to 0.64% though the absorption efficiency was significantly greater (98%), suggesting that almost all the dietary water absorbed from the flower is lost. Loss of water in insects may take place: 1. by defecation *via* feces, 2. by transmission through cuticle and 3. by evaporation through tracheal systems during respiration (WIGGLESWORTH, 1965). In *M. indica*, the loss of water through feces is negligible. Since the present experiment was conducted in an incubator, under a constant condition of temperature and humidity, which are not critical to *M. indica*, the possibility of transpiratory loss through the cuticle and absorption of ambient moisture is limited. Hence, the dietary water is likely to be mainly transpired through tracheal system. The loss of water through tracheal system in insect depends on the state of activity of insects (BURSELL, 1974; ARLIAN & VESELICA, 1979). During flight the demand for oxygen is increased. To meet the respiratory requirements the spiracular valves are opened and allow a high proportion of water to be lost from the tracheal system. In insects at rest, the spiracles may be kept closed and the proportion of water escaping through them may be correspondingly decreased (BURSELL, 1974). The maximal water loss through tracheal evaporation,

TABLE 1. Dietary water budget of *Mylabris indica*. Each value represents the average of 6 replicates.

Sex	Weight range (mg wet wt)	Feeding schedule (mg of fresh flower/month)	water intake (mg)	water loss via feces (mg)	water absorbed (mg)	water retained (mg)	water transpired (mg)
	42 \pm 2	2468.8	2119.8	42.9	2076.9	15.5	2061.4
Male	104 \pm 3	4074.6	3498.6	94.6	3404.0	10.3	3393.7
	220 \pm 3	4598.1	3948.1	107.1	3841.0	17.3	3823.7
Average		3714	3189	85	3104	14	3090
	42 \pm 2	2758.9	2368.9	44.0	2324.9	24.0	2300.9
Female	104 \pm 3	4371.7	3753.7	91.7	3662.0	14.0	3648.0
	220 \pm 3	4569.8	3923.8	115.3	3808.5	18.0	3790.5
Average		3898	3349	84	3265	19	3246

TABLE 2. Rate and efficiency of dietary water intake, absorption, retention and transpiration in *Mylabris indica*. Values are given in mg/g live weight/day. Results are based on the average performance of 6 replicates.

Sex	Weight range (mg wet wt)	Intake	Absorption	Retention	Transpiration	Absorption efficiency (%)	Retention efficiency (%)
	42 \pm 2	1.36	1.34	0.010	1.330	97.98	0.75
Male	104 \pm 3	1.05	1.03	0.003	1.027	97.29	0.30
	220 \pm 3	0.57	0.56	0.002	0.558	97.30	0.45
Average		0.99	0.98	0.005	0.972	97.52	0.50
	42 \pm 2	1.40	1.37	0.014	1.356	98.10	1.03
Female	104 \pm 3	1.11	1.09	0.004	1.086	97.60	0.42
	220 \pm 3	0.56	0.55	0.003	0.547	97.10	0.47
Average		1.02	1.00	0.007	0.996	97.60	0.64

than by cuticular transpiration, during flight activity, has been reported for locusts (MILLER, 1960; LOVERIDGE, 1968). In the light of these observations, it is suggested that the remarkably poor dietary water retention efficiency and maximal loss of ingested dietary water possibly through tracheal system in *M. indica* may be an adaptive mechanism for its active flight activity. This suggestion can be further supported by the fact that nonflying larval instars of *Danaus chrysippus* possess a comparatively higher water retention efficiency (30 to 54 %) (PANDIAN et al, 1978) than that obtained for *M. indica* (0.45 to 0.64%). The water reserve in insects also depends on the structure of the cuticle; insects with heavily sclerotized exoskeleton have relatively little water content (BURSELL, 1974; EDNEY, 1977). *M. indica* also has a phenolically tanned sclerotized exoskeleton. Water retaining capacity was found to be higher in females than in males of *M. indica*. Such a sexual variation in water retention efficiency has been reported in *Oryzaephilus surinamensis*; females having greater capacity than the males (ARBOGAST & MARGARET CARTHON, 1972).

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BIONOMICS OF *TRIOXYS* (*BINODOXYS*) SUBBA RAO & SHARMA, AN APHIDIID PARASITOID OF *APHIS CRACCIVORA* KOCH. VII. SEX RATIO OF THE PARASITOID IN FIELD POPULATION

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The predominance of the females over males was observed in each sample except in smaller ones ($n < 12$) which were collected either early or late in the season. The mean proportion of the females was 67% which significantly ($P = 0.001$, χ^2 -test) deviates from an expected sex ratio of 50 : 50. About 92% females in the field population were mated, reflecting the existence of an efficient mate finding and copulatory mechanisms. The sex ratio varies during the season. Males emerged earlier than the females from the diapausing mummies in the field. The results are explained and discussed.

(Key words: *Trioxys* (*Binodoxys*) *indicus*, *Aphis craccivora*, parasitoid, sex ratio, bionomics)

INTRODUCTION

The sex ratio of the parasitoid is of great biological significance from the point of view of the suppression of their hosts (the insect pests of the crops) (FLANDERS, 1967; SINHA & SINGH, 1979 a). In the past, FISHER (1958) postulated 1♂ : 1♀ ratio of the offspring for sexually reproducing organisms which was based on the assumption that natural selection acts to ensure equal parental investment in the production of either sex whereas HARTL & BROWN (1970) predicted a sex ratio of equal proportions of sexes in arrhenotokous species. SCHLINGER & HALL (1960, 1961), and HAMILTON (1974) have observed 1♂ : 1♀ sex ratio. However, in aphidiids (arrhenotokous species) distinct variations in female biased sex ratio has been reported (HAFEZ, 1961; HAMILTON, 1967; MACKAUER, 1976 a; RABASSE & BRUNEL, 1977; SHALABY & RABASSE, 1979). This paper relates to the study of the sex ratio of *Trioxys* (*Binodoxys*) *indicus* SUBBA RAO & SHARMA (Aphidiidae:

Hymenoptera), a competent parasitoid as a biocontrol agent against pigeon pea aphid, *Aphis craccivora* KOCH (Aphididae : Hemiptera) in the field observed during 1976-1979 and the same has been compared with the predicted sex ratio of aphidiids.

MATERIALS AND METHODS

General survey, collection and rearing of the pigeon pea aphid (host of *T. indicus*) has been described elsewhere (SINGH & SINHA, in press). Only those samples that yielded a minimum of ca. 50 emergents were considered for the sex ratio to preclude any drastic variation. However, smaller samples have also been analysed along with the larger ones to obtain an overall picture. The sex ratio was calculated as the proportion of the females (p) out of total number of adults (n) emerged after the pattern of MACKAUER (1976 a). Departures from an expected sex ratio ($p = 0.5$), were tested by χ^2 -test for goodness of fit. The proportion of mated females in the field population was also estimated by the counting of those females which produced female as well as male progenies. A seasonal variation in sex ratio was also recorded from the time of first emergence in December, 1978 till the end of the season (April, 1979).

RESULTS AND DISCUSSION

The predominance of females over males was observed in each sample of *T. indicus* except in smaller ones ($n < 12$) which were collected either early or late in the season (Table 1, Fig. 1). The mean proportion of females (\bar{p}) in 18 samples ($n \geq 50$) and in all the samples (54) taken together was 0.67 ± 0.07 and 0.60 ± 0.11 respectively which deviate significantly ($P = 0.001$, Table 2) from an expected sex ratio ($p = 0.50$). Earlier investigators have also reported such observation in many field collected mummies of aphidiids (HAFEZ, 1961; GUTIERREZ & BOSCH, 1970; PASS & PARR, 1971; CAMPBELL, 1974; MACKAUER, 1976 a). GUTIERREZ & BOSCH (1970) reported 1 : 1 sex ratio only when the adults were directly collected from the fields. The plausible explanation of the above findings may be the differential behavioural responses of the males and the females (MACKAUER, 1976 a). Earlier BOSCH *et al.* (1966, 1967) observed the migratory trend of females of *Aphidius*

smithi SUBBA RAO & SHARMA from the maturing fields and tried to explain this behaviour. However, the explanation advanced by the above authors does not seem to be plausible as the same factors which cause the migration of the females will tend the parasitoid to migrate from the neighbouring fields resulting in establishing the original sex ratio of the emergents in the field population. On the contrary, among the field collected adults (*T. indicus*) the predominance of the female is quite clear ($p = 0.83$, Table 2) and is significantly different from the sex ratio observed from mummies ($p = 0.67$). The smaller life-span of the male (*T. indicus*) probably appears to play a major role in contributing to the female biased sex ratio in the field population. However, differential mortality of developmental stages of the parasitoid (larval stages) and mummies related to sex (because of hyperparasitism, predation, pathogenic diseases, temperature, humidity etc., which may either operate singly or in conjunction) might possibly be affecting the sex ratio in

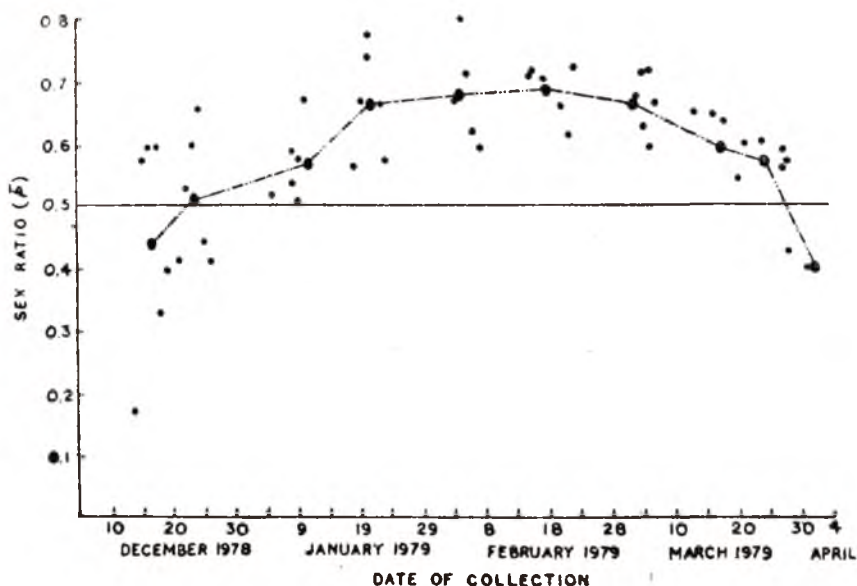


Fig. 1. Seasonal variation of the sex ratio of *Trioxys indicus* in the field population. ● ---- ● indicate average sex ratio of the respective week, ● — sex ratio of daily collection.

TABLE 1. Sex ratio in field population of *Trioxys indicus*, a parasitoid of *Aphis craccivora*.

	Samples 50 emergents	Total samples
Number of samples	18	54
Mean sample size	91.33 \pm 32.20	43.66 \pm 38.88
Expected sex ratio	0.50	0.50
Observed sex ratio	0.6569	0.6417
Mean sex ratio	0.6696 \pm 0.0722	0.6045 \pm 0.1146
99% confidence limit of mean sex ratio	0.6205 — 0.7187	0.5627 — 0.6463
Total χ^2	97.205*	114.388*
Pooled χ^2	80.548*	92.985*
Heterogeneity	16.657**	21.403**

1 Test of significance (H_0 : equal proportions of males and females)

* Significant, $P = 0.001$; ** Not significant.

TABLE 2. Sex ratio of *Trioxys indicus* in the field population as collected in adult form.

Year	Total number of <i>T. indicus</i>	Female	Male	Sex ratio	χ^2
1976-77	1179	1007	172	0.85	72.572*
1977-78	871	729	142	0.84	30.567*
1978-79	357	251	106	0.70	12.869**

1 Test of significance (H_0 : sex ratio is equal to 0.67, see Table 1).

* Significant, $P = 0.001$; **Significant, $P = 0.025$.

the field population, which needs further experimental confirmation.

The population of mated females of *T. indicus* is approximately 92% in the field (Table 3) which indicates that the parasitoid possesses great powers of mate finding mechanisms. The predominance of females in the field population is positively advantageous from the biological control point of view.

The sex ratio varies during the season. With its advancement (from mid December onwards) the sex ratio gradually builds up

and attains a peak, thereafter continues to decline by degrees till the last week of March and then sharply falls during the first week of April (Fig. 1). It was observed that the males emerge earlier than females from diapausing mummies which is in conformity with HAMILTON (1967) and HAMILTON (1974). This provides a fair opportunity for systematic mating resulting in a population composed of both sexes. This is significant biologically as the female aphidiids are known to possess a peculiar habit, i.e., they begin to oviposit shortly after their emergence provided the host(s) is available.

TABLE 3. Proportion of mated females of *Trioxys indicus* in field population.

Year	Total number females observed	Number of mated females	Proportion of mated females (%)	X ²
1976	36	31	86.1	0.9095*
1977	40	37	92.5	0.5833*
1978	67	62	92.5	0.6436*
1979	56	53	94.6	0.2619*

1 Test of significance (H₀ : all females are mated).

* Not significant

Had this interesting type of arrangement not been provided by nature, the ensuing progeny would have been only males. However, some of the earlier eggs laid by the females are bound to produce only male offspring either due to somewhat delayed mating or and newly mated females which are functionally virgin (MACKAUER, 1976 b). The higher sex ratio during the last week of January to first week of March (Fig. 1) may be explained on the basis of population dynamics of the host and the parasitoid. The population density of *A. craccivora* was higher in this period which results in the deposition of more fertilised eggs or a greater production of the females (SINHA & SINGH, 1979a). As the harvesting period of *Cajanus cajan* MILL. (host crop) approaches (from the 3rd week of March till the 1st week of April) the proportion of the males gradually increases, i.e., the sex ratio decreases (Fig. 1). This points out that the females enter diapause earlier than the males and in this respect *T. indicus* behave like *Aphidius rapae* (HAFEZ, 1961).

Therefore, it appears that the average sex ratio establishes around 0.65 (Table 1) and is significantly different from an equilibrium ratio of equal numbers of either of the sexes. The present findings strengthen the assumption of HARTL & BROWN (1970) that most of

the females of the population are mated and supports the evidence of MACKAUER (1976 a). The next supposition of HARTL & BROWN (1970) is that the population of parasitoids are essentially randomly mated. *T. indicus* have been found to be polygamous (1 mates with up to 5 females). In the laboratory, when a male was confined with a female (progeny of the same parents), it underwent a sequence of courtship behaviour which resulted in mating. After every hour the experiment was repeated by the same male with a 2nd, 3rd...6th successive females (randomly selected from the sample from which the 1st female was selected). It was observed that the mating with the 3rd female onwards was not only delayed but occurred in less individuals as well. However, the copulation with the 4th and 5th female (in succession) occurred only in a few replicates (Table 4). SUBRA RAO & SHARMA (1962) reported that a particular male mated with 12 females in succession. In the present study none of the males (out of 25 tested) mated with more than 5 females in succession. The females of *T. indicus* always prefer unmated mates for coitus. The sex ratio of the progeny of first three females (mated successively in a succession with a single male) averaged 0.70 ± 0.07 , 0.68 ± 0.13 and 0.64 ± 0.08 respectively with insignificant mean difference (Table 4). However, the

TABLE 4. Sex ratio of successive mated females by a single male of *Trioxys indicus*.

No. of successive mating	Total number of observations	No. of observations mating occurred	Sex ratio	Mean difference
1st	25	25	0.70 ± 0.069	..
2nd	25	25	0.68 ± 0.129	0.20*
3rd	25	21	0.64 ± 0.082	0.04*
4th	25	10	0.47 ± 0.099	0.17**
5th	25	3	0.29 ± 0.062	0.18***

*Not significant;

Significant, $P = 0.001$;*Significant, $P = 0.020$.

sex ratio of the offsprings of those females which were copulated with the males (already mated with 3 or 4 females in succession) averaged 0.47 ± 0.10 and 0.29 ± 0.06 respectively, differed significantly (*i.e.*, the 5th from the 4th and 4th from the first three females mated successively with a male) because males provide less sperm supply to fertilise desired number of eggs (SCHLINGER & HALL, 1960, 1961; WIACKOWSKI, 1962). A perusal of Table 4 indicates that successful mating of the pair having an exhausted male mate is discouraged. This type of behavioural response is probably the cause for the diminution of the proportion of males in the fields, thereby, the sex ratio is increased in the population. The sex ratio itself is not governed only by successful copulation of the females but also determined by several other extrinsic and intrinsic factors (FLANDERS, 1956, 1965, 1967). Some of the factors are the age, size and shape of the host (VINSON, 1976); density of host and parasitoid (LENGER, 1967; SINHA & SINGH, 1979 a); physical and physiological conditions of the host and the parasitoid, instinctive behaviour of the parasitoid (female) (SINHA & SINGH, 1979 a); rate of oviposition (FLANDERS, 1965); temperature (MOURSİ, 1946; FORCE

& MESSENGER, 1964) etc. The genetic mechanism of sex determination also influences the sex ratio as well (WHITING, 1943).

Most of the aphidiid wasps are known to deviate from the predicted sex ratio of equal proportions of males and females. The plausible explanation of such deviation is that the models of FISHER (1958) and HARTL & BROWN (1970) lack certain etho-ecological characteristics of the host-parasitoid systems. In spite of that they do provide a useful framework for testing observed sex ratios.

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* Not seen in original.

STUDIES ON A NUCLEAR POLYHEDROSIS OF RICE CASEWORM *NYMPHULA DEPUNCTALIS* GUEN. (PYRAUSTIDAE : LEPIDOPTERA)

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Studies on nuclear polyhedrosis of the larvae of the rice caseworm *Nymphula depunctalis* GUEN. (Pyraustidae: Lepidoptera) revealed that the infected larvae exhibited all the typical symptoms of nuclear polyhedrosis. Third, fourth and fifth instar larvae were highly susceptible while the sixth instar larvae were less susceptible to the infection. The LT_{50} values for third to sixth instar larvae ranged from 3.0766 to 6.5335 days. Adipose tissues, hypodermis and tracheal matrix cells were the major sites of virus infection. Polyhedral formation was also observed to a limited extent in cells of Malpighian tubules and wing buds. The polyhedra varied in size and shape. The rod shaped viral particles were arranged in bundles within the polyhedra. Polyhedra were completely soluble in weak solutions of NaOH, KOH and Na_2CO_3 . The thermal inactivation point of the virus was between 85 and 90°C. The virus was not infective to eleven species of alternate caterpillars tested. The virus could withstand weathering upto 48 hours without any loss of virulence. But further weathering caused a gradual loss of viral activity though it retained substantial infectivity upto 964 hours of weathering and by 192 hours complete inactivation occurred.

(Key words: nuclear polyhedrosis, *Nymphula depunctalis*)

INTRODUCTION

The rice caseworm *Nymphula depunctalis* GUEN. causes severe damage to rice crop in its early stages. JACOB *et al.* (1978) reported the occurrence of a nuclear polyhedrosis in this insect. Information gathered on the nature of the pathogen and on the host-pathogen relationships are presented in this paper.

MATERIALS AND METHODS

The larvae used in these studies were reared in the laboratory on paddy (*Oryza sativa* L.) leaves. A purified concentrated suspension of polyhedra isolated from the diseased larvae of *N. depunctalis* and diluted to contain 42×10^5 polyhedra per ml of distilled water formed the infective material. Teepol (0.1 per cent) was used as wetting agent. Larval inoculations were done by the spot feeding technique (JACOB, 1972). Five microlitres of the polyhedral suspension were applied to each spot and the larvae which had consumed the treated leaf spot completely within 2

hours were transferred to a new virus free seedling planted in pots. Control larvae were fed similarly on spots of distilled water containing 0.1 per cent teepol only.

Susceptibility of different instars of the larva was assessed as indicated by JACOB & SUBRAMANIAM (1972). Histopathological studies were made by inoculating third instar larvae as described by JACOB & SUBRAMANIAM (1973). Dissolution of polyhedra in alkalies was studied by the method of PAWAR & RAMAKRISHNAN (1972). Thermal inactivation point of the virus was studied as described by LATHIKA & JACOB (1974).

Cross infectivity to alternate species of lepidopterous larvae was determined by feeding them for 24 hours on their respective host plant leaves contaminated with the polyhedral suspension. In food consumption studies third instar larvae were inoculated with the polyhedral suspension as described above. At one day intervals the leaf area fed by each caterpillar was assessed by tracing it on a graph paper and the total area fed was estimated. These observations were taken for 7 days.

To study the effect of weathering on the virulence of the virus, one ml of the polyhedral inoculum was painted uniformly on both sides of leaves of paddy planted in pots. The pots were then kept outside exposed to field conditions. The virus activity of treated leaves was assayed against third instar larvae at 24 hour intervals until there was no larval mortality due to virus infection.

RESULTS

Symptomatology

The infected, third, fourth and fifth instar larvae turned pale 3 days after ingestion of the virus. The larvae became lethargic, less responsive to tactile stimuli and developed symptoms of anorexia and stopped feeding in 3–5 days. Larvae infected in the early instars appeared smaller in size as the disease progressed. Inability to construct new cases was also noticed in many cases. Towards the advanced stages of the disease the whole body acquired a whitish colour and the cuticle became highly fragile and it ruptured liberating the liquefied body contents containing the polyhedral inclusion bodies. Most of the larvae came out of their cases before death. The incubation period varied from 3–8 days. Dissection of the infected

larvae showed that the fat body was opaque white or porcelain white in appearance. Many of the fifth and sixth instar larvae completed the larval stage, but some of them died in the pupal stage. In infected pupae the tissues were liquefied.

Larval susceptibility

Results presented in Table 1 show that as the stage of larvae at inoculation advanced there was a decrease in the mortality caused by the virus infection and a prolongation of the incubation period. Third and fourth instar larvae showed high susceptibility to virus infection. Fifth instar larvae also showed a fairly high susceptibility with 74 per cent mortality. The sixth instar larvae were however less susceptible. The LT_{50} values for third to sixth instar larvae ranged from 3.0766 to 6.5335 days.

Histopathology

No signs of infection were visible in any of the tissues in 24 hours after inoculation. The first noticeable evidence was an enlargement of the nuclei of the infected fat cells after 48 hours of inoculation. By 72 hours the nuclei of infected fat cells had enlarged

TABLE 1. Susceptibility of different larval instars of *N. depunctalis* to infection* by NPV.

Instar of larvae	Incubation period (days)		per cent larval mortality due to		per cent pupation
	Range	Mean	Polyhedrosis	Other causes	
III	3–7	4.34	100
IV	4–8	5.56	100
V	4–8	5.64	74	..	26
VI	4–8	6.10	58	..	42

1. 50 larvae of each instar were used.
2. There was no mortality due to virus in control.

further and a few nuclei showed the presence of polyhedra. The epidermis also showed slight enlargement of the nuclei. At 96 hours after inoculation polyhedra were visible in many cells of the fat body, hypodermis and tracheal epithelium. Polyhedral formation was also observed in wing buds and Malpighian tubules on a limited scale. By 120 hours the infection had spread throughout the fat body and the tissues appeared disintegrated in many regions.

Size and shape of polyhedra

Electron micrograph (Fig.1) of polyhedra showed variation in size and shape. The rod shaped virions were arranged in bundles.

Effect of alkali on polyhedra

It may be seen from Table 2 that the polyhedra dissolved completely in 0.1 and 0.2 per cent solution of sodium hydroxide within 1 minute. But potassium hydroxide at 0.1 and 0.2 per cent concentration re-

quired 3 and 2 minutes respectively to dissolve the polyhedra completely. With 5 per cent sodium carbonate the dissolution of polyhedra was achieved in 20 minutes while the 10 per cent solution required only 15 minutes to produce the same result.

Thermal inactivation point of the virus

The results (Table 3) reveal that infectivity of the virus was not affected by exposure to temperatures upto 60°C for 10 minute, but it started declining when the temperature was raised to 70°C and above. The virus did not show any infectivity when subjected to a temperature of 90°C. These indicate that the thermal inactivation point (TIP) of the virus lay between 85 and 90°C.

Cross infectivity

Results of cross transmission studies showed that the NPV of *N. depunctalis* was not infective to larvae of *Hymenia recurvalis* Fb., (Pyraustidae), *Sylepta drogata* Fb., (Pyraustidae), *Cnaphalcerosis medinalis*

TABLE 2. Effect of different alkalis on polyhedra of *N. depunctalis*.

Time taken for dissolution of polyhedra (minute)	NaOH(%)		KOH (%)		Na ₂ CO ₃ (%)	
	0.1	0.2	0.1	0.2	5.0	10.0
1	—	—	+	+	+	+
2	—	—	+	—	+	+
3	—	—	—	—	+	+
4	—	—	—	—	+	+
5	—	—	—	—	+	+
10	—	—	—	—	+	+
15	—	—	—	—	+	—
20	—	—	—	—	—	—

(+) Polyhedra present.

(—) Polyhedra absent.

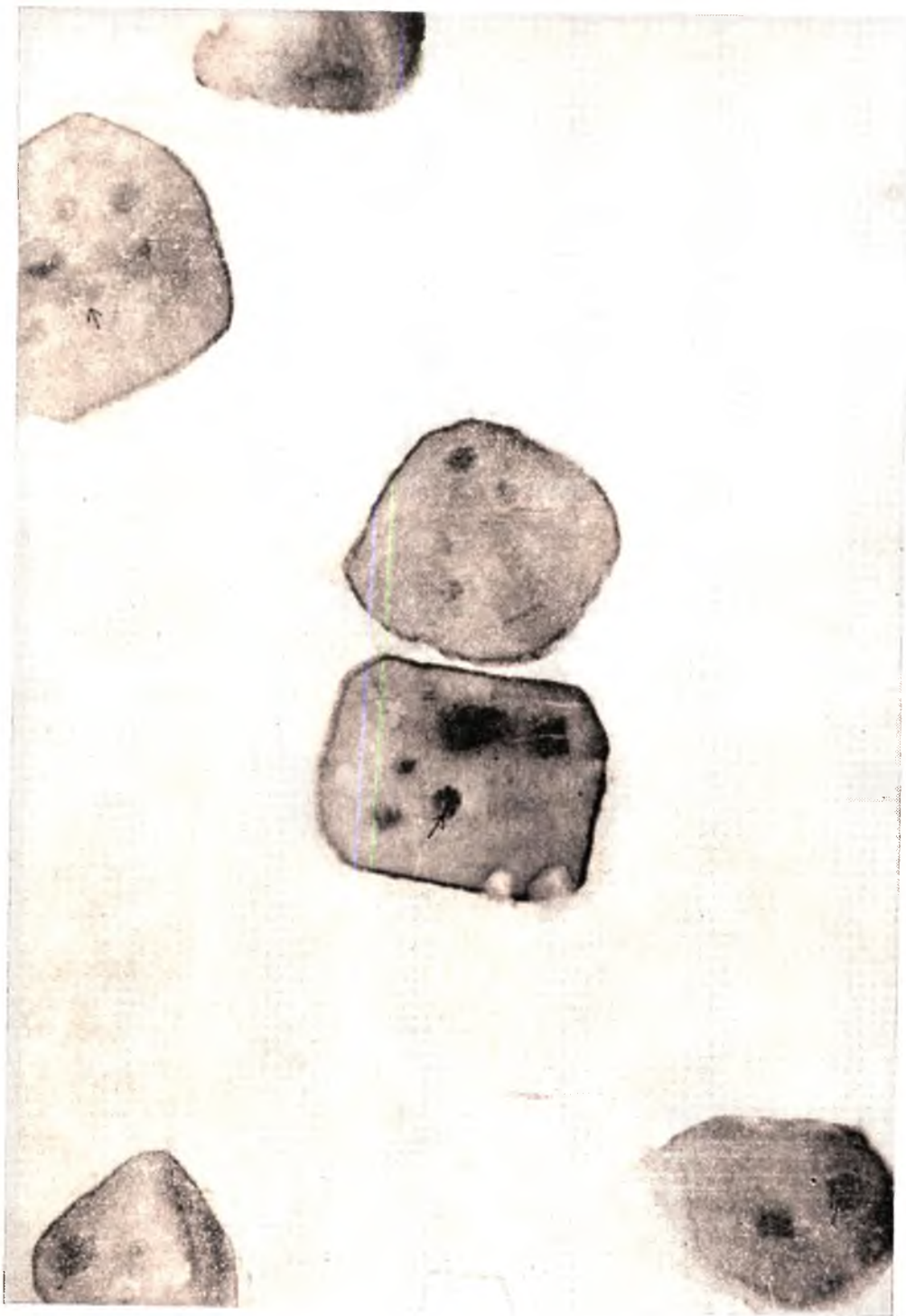


Fig. 1. Electron micrograph of polyhedra isolated from NPV infected larvae of *N. depunctalis* Guen 34,000 X.

GUEN. (Pyraustidae), *Antigastra catalaunalis* D. (Pyraustidae), *Pasara basalis* FB. (Pyralidae), *Margaronia indica* SOUND (Pyralidae), *Glyphodes marginata* (Pyralidae), *Nephantis serinopa* MEYR. (Cryptophasidae), *Spodoptera mauritia* BOISD. (Noctuidae), *Spodoptera litura* FB. (Noctuidae) and *Plusia peponis* F. (Noctuidae).

Food consumption

The mean area of paddy leaves consumed by healthy and NPV infected larvae at different intervals after inoculation are summarised in Table 4. It may be seen that there was no marked difference in the leaf consumption by healthy and infected larvae during the first 24 hours of ingestion of the virus. But the mean area of paddy leaf consumed by the diseased larvae at all intervals after one day of inoculation was significantly lesser than that by comparable healthy larvae, the percentage of reduction in leaf area fed being 22.24,

47.52, 65.90, 75.86 and 86.19 on second, third, fourth, fifth and sixth days respectively.

Effect of weathering of polyhedra of

N. depunctalis on the virulence of the virus.

The results presented in Table 5 show that the infectivity of the virus was unaffected by exposure upto 48 hours but further exposure to environmental conditions reduced its infectivity. Analysis of the data by test showed that there was significant difference in the larval mortality caused by the virus exposed to 9 hour and that caused by the virus exposed to 72 hours or more. However it retained substantial infectivity upto 96 hours of weathering causing 62 per cent larval mortality. The mortality decreased to 10 per cent by 168 hours of weathering. The LT_{50} values for the different intervals of weathering upto 72 hours did not show much variation but showed an increase

TABLE 3. Effect of different temperatures on the infectivity of the NPV of *N. depunctalis* when exposed for 10 minutes.

Temperature (°C)	No. of larvae inoculated	Incubation period in days (Range)	% larval mortality due to polyhedrosis	Pupation %	Adult emergence %
50	25	3-7	100
60	25	3-7	100
70	25	3-8	80	20	20
80	25	3-8	60	40	40
85	25	3-8	20	80	80
90	25	100	100
100	25	100	100
Control (untreated virus)	25	3-7	100
Control (without virus)	25	100	100

TABLE 4. Area of paddy leaf consumed by healthy and virus infected larvae of *N. depunctalis*.

Post inoculation period in days	Leaf area consumed per larvae in mm ² (Mean \pm SE)		Per cent increase (+) decrease (-) over healthy
	Healthy	Diseased	
1	75.40 \pm 2.90	74.55 \pm 2.30	-1.13
2	99.53 \pm 3.48	77.40 \pm 3.10	-22.24
3	113.66 \pm 2.84	59.66 \pm 5.05	-47.52
4	120.66 \pm 2.45	41.15 \pm 6.03	-65.90
5	124.26 \pm 1.46	30.00 \pm 7.37	-75.86
6	127.80 \pm 1.02	17.66 \pm 2.24	-86.19
7	136.20 \pm 1.17

*Mean of 15 estimations

TABLE 5. Effects of weathering on the virulence of the virus.

Effect	Control (untreated)	Larval response to virus weather a few different periods								
		0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	192 hrs
Per cent mortality	0	100	100	100	80	62	40	18	10	0
Mean no. of days to death	..	4.38	4.34	4.38	4.37	4.46	4.35	4.50	4.40	..
Per cent pupation	100	0	0	0	20	38	60	82	90	100
Per cent adults	100	0	0	0	20	38	60	82	90	100
LT ₅₀ (days)	.. 2.8028	3.9068	2.8006	3.2961	5.3952

thereafter. The LT₅₀ values for 120, 144 and 168 hours of exposure could not be calculated as the larval mortality due to the virus was less than 50 per cent.

DISCUSSION

The symptoms of the infection by NPV in the larvae of *N. depunctalis* resembled those described for NPV of other lepid-

pteran larvae as reviewed by AIZAWA (1963) and SMITH (1967). Many of the larvae inoculated in the fifth and sixth instars completed the larval stage but some of them died in the pupal stage. Similar observations were made by STAIRS (1965b) in *Galleria mellonella* and VAIL & HALL (1969) in *Trichoplusia ni*. The young larvae were found to be more susceptible to the virus

than older ones. Such observations were made by TANADA (1956) MORRIS, (1962), STAIRS (1956a), and JACOB & SUBRAMANIAM (1972). It is a case of maturation immunity which according to IGNOFFO (1966) is partly due to the normal increase in body weight of the host which might dilute a constant viral dose.

Histopathological symptom in the larvae of *N. depunctalis* generally resembled those described for NPV in other lepidoptera. The hypodermis, tracheal matrix and fat body were the major sites of infection. Polyhedral formation was also observed to a limited extent in the Malpighian tubes and wing buds. Infection of these latter tissues have been previously reported in a few instances (STAIRS, 1965b; ADAMS *et al.*, 1968; HAMM, 1968; JACOB & SUBRAMANIAM, 1973). The virus rods were arranged only in bundles within the polyhedra. Thus it may be grouped under the multiple embedded virus (MEV) type. The virus rods occurred singly (single embedded virus-SEV) in *Heliothis zea* (GREGORY *et al.*, 1969) and in *H. armigera* (JACOB & SUBRAMANIAM, 1972). But in many cases the virus rods occurred singly and in bundles (ADAMS *et al.*, 1968, JACOB & SUBRAMANIAM, 1972; NAIR & JACOB, 1975).

In common with other polyhedral viruses the polyhedra of *N. depunctalis* dissolved in solution of NaOH, KOH and Na_2CO_3 . It is known that the degree of resistance towards different alkalies varied with polyhedra from different insects. In its reaction towards weak solutions of NaOH and KOH, the polyhedra of *N. depunctalis* closely resembled those of *S. mauritia* (LATHIKA & JACOB, 1974a) and *Diacrisia obliqua* (JACOB & THOMAS, 1974). The polyhedra were slightly resistant to Na_2CO_3 but less resistant than polyhedra from *Pterolocera amplicornis* (DAY *et al.*, 1953).

The TIP of NPV of *N. depunctalis* is seen to be between 85 and 90°C. It exceeded the general limit of 80°C reported for other inclusion viruses (BERGOLD, 1958; AIZAWA, 1963; HUGER, 1963). However, it was less heat-tolerant than the NPV of *S. litura*, *S. mauritia* and *P. ricini* (PAWAR & RAMAKRISHNAN, 1971; LATHIKA & JACOB, 1974b; NAIR & JACOB, 1975).

The cross-infectivity studies indicate a fairly high degree of host specificity of the virus as was common to most of the nuclear polyhedrosis virus (SMITH, 1967).

The observation that virus infection caused significant reduction in food consumption from the second day of ingestion of polyhedra indicated that though the virus infected larvae were killed only slowly the crop loss due to the pest incidence would be reduced significantly. Insect viruses generally did not retain their infectivity for long under field conditions (IGNOFFO, 1966; CANTWELL, 1967). The present studies showed that the NPV of *N. depunctalis* could withstand exposure for 48 hours without any loss of infectivity and retained substantial infectivity even after 96 hours of weathering, but was almost non-infectious after 168 hours. Perhaps temperature along with other factors like ultraviolet radiation may be causing the deactivation as suggested by IGNOFFO (1966) and BULLOCK (1967).

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ISOLATION OF A BACTERIAL PATHOGEN FROM THE COCONUT PEST *ORYCTES RHINOCEROS* L.

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The Indian coconut beetle, *Oryctes rhinoceros* L. (Dynastinae, Scarab. Col.) is a serious pest on coconut. A bacterial pathogen which has been identified as *Acinetobacter calcoaceticus* has been isolated and has been confirmed as a potential agent for the biological control of *Oryctes rhinoceros* grubs. The pathogenic effect of the bacteria and its culture filtrate on the larval stages were studied.

(Key words: *Oryctes rhinoceros*, bacterial pathogen, coconut pest, *Acinetobacter calcoaceticus*, biological control agent, pathogenic effect)

INTRODUCTION

Several strains of *Bacillus* are known to cause milky disease in the larval stages of insects belonging to Scarabaeidan family (BEARD, 1956; DUTKY, 1940; KAWANISHI *et al.*, 1974; TASHIRO & WHITE, 1954; WHITE, 1947; WILLE, 1956; TASHIRO, 1957). However, no information is available for the occurrence of such a disease in *Oryctes rhinoceros* L. grubs. In the present communication, we report the isolation of a bacterial pathogen *Acinetobacter calcoaceticus*, infecting the grubs of the coconut beetle, *Oryctes rhinoceros* L.

MATERIALS AND METHODS

Larvae of *Oryctes rhinoceros* L. of different instars were collected from manure heaps. Earthenware pots (15 inches height and 8 inches diameter) filled with cowdung having 40 to 50% moisture holding capacity were used to maintain the grubs. Sterile distilled water was sprinkled on alternate days to maintain the moisture of the feeding material. Many investigators (TASHIRO & STEINKRAUS, 1966) injected the spores or the vegetative cells of the pathogen into the hemocoel, to test the pathogenic effect on scarabaeidan beetle grubs and this procedure has come into routine use in many laboratories. Injec-

tions were made according to the procedure of JULIAN & HALL (1968) with an Agla microinjector*. Care was taken to avoid the puncture of gastrointestinal tract. Control grubs were injected with 0.1 ml of sterile phosphate buffer (0.05 M, pH 7.0), sterile distilled water or sterile medium. After inoculation, five grubs were placed in each pot and were incubated at room temperature ($30 \pm 2^\circ\text{C}$). The inoculated grubs were examined for gross symptom of infection at various time intervals. Hemolymph samples were collected and examined microscopically for the presence of the pathogen and for disease development for a period of five weeks.

In an attempt to isolate a pathogenic bacterium from a naturally infected grub, collection of grubs were made from coconut growing areas and from the manure heaps. Grubs with dark brown and blue color, presumably showing symptoms of natural infection were collected from the manure heaps. The diseased grubs were collected in the vicinity of Madurai, Tamilnadu. Only the 3rd instar of the grubs were found to be with natural infection and they were collected in the month of September 1977. Following the procedure of JULIAN *et al.* (1970), these infected grubs were washed free of soil with 45 to 50°C tap water and then rinsed for 30 sec with 5% sodium hypochlorite. The hemolymph was aseptically collected from the infected, surface sterilized larva by snipping off one of the fore legs. It was then suitably diluted and plated on nutrient agar plates (0.5% peptone, 0.3% beef extract, 0.2% yeast extract, pH 7.0; for solid medium 2% agar was used). Pure cultures

* Wellcome Reagents, Limited, England.

were obtained from single cell colonies and each isolate was tested for its ability to infect the grub. Growth in nutrient broth was measured in shake cultures (180 to 200 rpm at $30 \pm 2^\circ\text{C}$). Cells were harvested from appropriate phases of growth, washed and resuspended in sterile buffer (0.05 M, pH 7.0). The concentration of the cells was adjusted to the desired level before injection to the grubs.

For oral feeding the larvae were anaesthetised with ether and a sterile rubber tube was inserted through the mouth without damaging the alimentary canal. The bacterial suspension was forced through the rubber tube with the help of a syringe. The control grubs received the same amount of sterile distilled water and sterile phosphate buffer (0.05 M, pH 7.0). The forcefed grubs were incubated and examined for development of disease symptoms as described before.

For voluntary oral feeding experiments, the bacterial cell suspension was mixed with known weight of manure pit soil. Grubs were then incubated in the bacteria mixed soil and they were periodically examined for the development of disease symptoms. The culture filtrate (CF) was obtained from shake cultures. The stationary phase culture was centrifuged at $10,000 \times g$ for 20 minutes. The bacteria-free supernatant was then passed through a millipore membrane filter (0.45 μ , Millipore Corporation, USA) and 0.1 ml aliquots of this was injected into hemocoel of the grubs.

For each experiment a total of 300 grubs were used and for the control experiments, 25 grubs were tested. For all the experiments only the 3rd instar grubs were used. This is because of its larger life period when compared to the other instars.

Symptoms of infection

After the bacterial infections the grubs become blue in color. The blue color development started from the posterior end of the grub and it extended to the anterior end. The grubs lost their rigidity and at that stage they were very sluggish and stopped feeding. Before death the larvae moved deeper into the feeding material and they were watery inside. After the death of the grub hemolymph oozes out from the body of the grub.

RESULTS

Injection of 10^6 cells/grub with the pathogenic bacterium caused death of the grub within 6 to 12 hrs (Table 1). To find out

whether the death of the grub was due to the bacterial pathogen that was injected, hemolymph was collected before the death of the grub and plated on nutrient agar. The colonies that developed were compared with the original isolate following the description given in the Bergy's manual. Death of the grubs could also result from injection of the culture filtrate into the hemocoel. Development of blue color and death of the grub was observed 9 to 14 hrs after injection of the culture filtrate (Table 2). When it was possible to induce symptoms and death with culture filtrate it was tested for its chemical tolerance with heat. The culture filtrate was kept for 30 min at 80°C in a constant temperature water bath and 0.1 ml of the culture filtrate was tested for its pathogenic effect by injecting into the hemocoel. The grubs were found to die within 9 to 14 hr after developing a blue color (Table 2). This suggests that the metabolic product present in the culture filtrate of the bacterium was heat stable and was lethal to the grubs.

Infectivity test was also done by mixing the bacterium with unsterilized manure heap soil. The pathogenic effect was observed with blue color development after 25 to 30 days. Above 10^8 cells/g of manure heap soil, the infectivity was observed as 56%. Mixing the culture filtrate with the manure heap soil results no infectivity (Table 3). This pathogenic bacterium was identified as *Acinetobacter calcoaceticus* by the Culture Collection Centre, United Kingdom. In all the experiments the control grubs showed no sign of death. They pupated normally and normal adults emerged.

DISCUSSION

In an attempt to isolate a disease causing pathogenic organism for the *Oryctes rhinoceros* L. grubs, a gram negative coccoid, *Acinetobacter calcoaceticus* was obtained. These organisms are commonly found in soil

TABLE 1. Influence of infection of *A. calcoaceticus* on *O. rhinoceros* grubs.

Concentration of cells/grub*	Time taken for death	% of grubs died
10 ⁵	6 to 10 hrs	20%
10 ⁶	6 to 9 hrs	90%
10 ⁷	6 to 9 hrs	99%
Control	..	No death

*The percentage of grubs died was determined by testing 300 grubs for each class.

TABLE 2. Influence of culture filtrate of *A. calcoaceticus* on *O. rhinoceros* L. grubs.

Treatment*	Volume of the culture filtrate	Time taken for death	% of grubs died
Millipore passed culture filtrate	0.1 ml	9 to 12 hrs	80%
	0.2 ml	9 to 10 hrs	100%
CF heated at 80°C for 30 min	0.1 ml	9 to 14 hrs	60%
	0.2 ml	9 to 12 hrs	80%
Control	0.2 ml of sterile medium	..	No death

*Percentage of grubs died was determined by testing 300 grubs for each class.

and water (TAYLOR & JUNI, 1961). The pathogenic bacterium *Acinetobacter calcoaceticus* is aerobic, gram negative, non-motile, catalase positive and oxidase negative. *Acinetobacter calcoaceticus* resemble saprophytic pseudomonas and able to use any one of a large number of organic compounds as a carbon and energy source (JUNI, 1978). It has been demonstrated that some strains of *Acinetobacter* can be the causative agents of diseases in animals (DALY *et al.*, 1962; GARDNER *et al.*, 1970; HENDERSON, 1965; HERMAN & JUNI, 1974). However, the pathogenic nature of this bacterium in insects has not been reported so far.

The development of the blue color after infection has not been reported so far. However, it was reported that, while dropping the grub from a higher place leads to the rupturing of the foregut wall and mixing up of the gut juices with the hemolymph produces the blue color and it was named as blue diseases (BEDFORD, 1968). In cases where the infection is due to *Bacillus thuringiensis*, the toxic parasporal body was broken by the alkaline and midgut contents of the susceptible insects and the activated toxic material then affects the permeability of the gut epithelium, allowing the alkaline contents of the midgut to leak into the hemocoel, raising the pH of the blood and leads to the death of the larvae (WOOD, 1974). Probably

TABLE 3. Influence of *A. calcoaceticus* and its culture filtrate on *O. rhinoceros* L grubs when mixed with manure heap soil.

Concentration of cells/g of soil*		Number of days taken for death	% of infection
<i>A. calcoaceticus</i>	10 ⁸	25 to 35	10%
	10 ⁹	25 to 35	15%
	10 ¹⁰	25 to 30	56%
Culture filtrate	100 ml/kg of soil	..	no apparent influence
Control	without CF and without bacterial cells	..	no death

*Percentage of grubs was determined by testing 300 grubs for each class

the toxic metabolite (unknown) of the *Acinetobacter calcoaceticus* alters the permeability of the gut wall and allows the gut juices to mix with the hemolymph to produce the blue disease. The use of this gram negative coccobacillary form as a biological control agent remains to be seen, since its survival value in different types of manure pits remains to be elucidated. The mixing up of the CF with unsterilized cowdung results without pathogenic effect. This may be due to the denaturation of the CF in the cowdung.

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COMPARISON OF CULTURAL AND CHEMICAL METHODS FOR THE CONTROL OF SORGHUM SHOOT FLY*

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Field trials were conducted to compare the cultural method of using higher seed rate and removing and destroying damaged seedlings with chemical control recommended for the control of sorghum shoot fly.

The data on oviposition showed that significantly more eggs were laid on seedlings emerging from carbofuran treated seed. This is attributed to relatively healthier plant stand and dark green colouration of the seedlings. The dead hearts formed in treatments where untreated seed was used were significantly higher than those where carbofuran treated seed was used @ 8 and 10 kg ha; these two treatments were, however, not significantly different from the treatment where 6:4 mixture of carbofuran treated and untreated seed was used. Yields of grain and fodder were also much higher in treatments where carbofuran treated seed was used.

It is concluded that the cultural practice of higher seed rate and removing damaged seedlings was not effective under heavy shoot fly infestation condition. However, 6:4 mixture of carbofuran treated and untreated seed used @ 10 kg ha can reduce the shoot fly damage considerably and result in yields comparable to only carbofuran seed treatment.

(Key words: control methods, cultural, chemical, sorghum shoot fly)

INTRODUCTION

One of the oldest recommendations for controlling sorghum shoot fly *Atherigona soccata* (RONDANI), is based on cultural practice of using higher seed rate and removing and destroying damaged seedlings (FLETCHER, 1914; AYYAR, 1932).

In recent years a number of investigators have developed and recommended suitable methods for the control of this pest. By and large most of these methods include the use of different insecticides (JOTWANI & SUKHANI, 1968; THOBBI *et al.*, 1968; RAO & RAO, 1971; USMAN, 1972; JOTWANI & YOUNG, 1972) which are so expensive that recommendations made have not been generally adopted by the average sorghum grower. It was considered desirable to compare the

chemical method with the cultural method and if possible, to develop integrated approach of utilising both cultural and chemical methods, the ultimate aim being to develop a technique for effective and economical control of the shoot fly which may be acceptable to an average sorghum grower.

MATERIAL AND METHODS

The experiments were carried out in the farm area of the Indian Agricultural Research Institute, New Delhi during *Kharif*-1974 and 1975. The trials were laid out in randomized block design; there were 6 treatments each replicated 4 times. Seed of hybrid CSH-1 was sown in both the experiments. To ensure high shoot fly infestation, sowings of the experiments were delayed for about a fortnight and spreader rows were sown around the experimental plots 2 weeks earlier. Fertilizers were applied at the recommended doses *viz.* 100 kg of nitrogen (split dose) and 60 kg of P_2O_5 . The excess plants were thinned two weeks after germination by removing shoot fly infested and healthy seedlings, keeping plant to plant distance at 15 cm.

*A part of Ph.D. thesis submitted by the senior author.

All plots, including control, were protected from borer damage (*Chilo partellus* (SWINHOE)) by applying endosulfan 4% granules in leaf whorls 8, 10 and 12 kg/ha on 20th, 30th and 40th day after germination. At seed setting, all experimental plots were treated with endosulfan @ 0.5 kg a.i./ha for protecting against midge and earhead worms damage. The first application was given at 90 per cent earhead emergence and second, 5-6 days after the first application. The sprayings were directed on the earheads only.

The number of eggs laid by shoot fly was recorded 2 weeks after germination and dead hearts were recorded 2 and 3 weeks after germination. Yields of fodder and grain were recorded at harvest.

RESULTS AND DISCUSSION

The data collected during the two seasons were subjected to analysis of variance. The results are presented in Table 1.

Oviposition responses of shoot fly:

The data collected on number of eggs laid by shoot fly on 10 randomly selected plants, 2 weeks after germination, revealed that during *Kharif*-1974 oviposition by shoot fly was significantly lower in the treatments where untreated seed was sown @ 8, 10 and 12 kg/ha. The treatments where 6:4 mixture of carbofuran treated and untreated seed was used occupied the intermediate position. Significantly more eggs were laid on seedlings emerging from carbofuran treated seed.

The results obtained in *Kharif*-1975 showed similar trend. Maximum eggs/ 10 plants (21.50) were laid in treatments where carbofuran treated seed was sown @ 10 kg/ha and minimum (8.50) in the treatment where untreated seed was sown @ 10 kg/ha.

The differences in oviposition can be attributed to healthier plant stand and dark green colour of the seedlings emerging from carbofuran seed treatment which possibly attracted the shoot fly for oviposition.

Dead heart formation in main shoots:

During *Kharif*-1974 the dead heart formation due to sorghum shoot fly was signi-

ficantly lower in treatments where carbofuran treated seed was sown @ 8-10 kg/ha. These two treatments were at par with the treatment where 6:4 mixture of carbofuran treated and untreated seed was sown. Dead hearts were significantly higher in the plots where untreated seed was sown. The differences between three seed rates were not statistically significant.

Similar results were obtained during *Kharif*-1975 also. Statistically there were two distinct groups, the first group consisted of all the treatments where carbofuran treated seed was used either alone or as mixture with untreated seed and the second group consisted of all the treatments where only untreated seed was used. Maximum dead hearts (75.54%) were recorded in the treatment where untreated seed was sown @ 8 kg/ha and minimum (5.23%) in the treatment where carbofuran treated seed was sown @ 8 kg/ha.

The results of two field experiments have indicated that the recommended cultural control of using higher seed rate followed by uprooting and destroying the infested seedlings did not reduce the dead heart formation. The carbofuran treated seed sown @ 8 and 10 kg/ha and mixture of carbofuran treated and untreated seed (6:4) sown @ 10 kg/ha showed significantly less dead hearts. These three treatments were statistically at par with each other.

Grain and fodder yields:

In *kharif*-1974, carbofuran treated seed sown @ 8 and 10 kg/ha and 6:4 mixture of carbofuran treated and untreated seed sown @ 10 kg/ha resulted in significantly higher grain yield as compared to other treatments where only untreated seed was used. Maximum grain yield (46.71 Q/ha) was obtained in carbofuran treated seed sown @ 10 kg/ha and minimum (13.85 Q/ha) in untreated seed sown @ 8 kg/ha.

TABLE 1 Effect of different seed rates of carbofuran treated and untreated seeds on the infestation and damage by a sorghum shootfly.

Treatments	Khari/-1974				Khari/-1975			
	Av. no. of eggs/ 10 plants	Av. % dead hearts upto 3 weeks	Av. yield Q/ha Grain	Av. yield Q/ha fodder	Av. no. of eggs/ 10 plants	Av. % dead hearts upto 3 weeks	Av. yield Q/ha Grain	Av. yield Q/ha Fodder
Carbofuran treated seeds (5% a.i) sown @ 8 kg/ha	9.75	10.7 (18.81)	38.15	101.50	17.50	2.1 (5.23)	38.20	250.75
Carbofuran treated seeds (5% a.i) sown @ 10 kg/ha	10.25	7.1 (15.36)	46.71	111.50	21.50	1.3 (5.43)	45.53	239.75
Seed mixture of 6 kg carbofuran treated seeds 5% (a.i) and 4 kg untreated seeds	7.75	14.9 (22.68)	27.89	88.25	15.50	4.8 (12.62)	38.87	208.50
Untreated seeds sown @ 8 kg/ha	5.75	70.8 (57.93)	13.85	59.75	10.25	91.6 (75.54)	5.53	44.00
Untreated seeds sown @ 10 kg/ha	5.75	73.8 (59.29)	18.30	64.00	8.50	85.5 (69.52)	6.71	46.25
Untreated seeds sown @ 12 kg/ha	5.75	74.8 (60.12)	18.82	73.75	8.75	88.7 (72.42)	6.57	48.50

Figures in parentheses are transformed values = Arcsin Percentage

S Em ±	0.58	(2.42)	1.7	7.3	0.84	4.50	2.33	9.71
CD at 5%	1.74	(7.42)	5.11	21.99	2.54	13.55	7.01	29.26
CD at 1%	2.41	(10.26)	7.07	30.41	3.51	18.74	9.70	40.46

Similar trend was observed in the case of fodder yield. Maximum fodder yield (111.50 Q/ha) was obtained in treatment where carbofuran treated seed was sown @ 10 kg/ha and minimum (59.75 Q/ha) where untreated seed was sown @ 8 kg/ha.

During *Kharij*-1975 based on statistical differences the grain yield showed two distinct groups, the first comprising of untreated seed sown @ 8, 10 and 12 kg/ha which gave significantly lower yield as compared to second group of treatments where carbofuran treated seed was sown @ 8 and 10 kg/ha and also where 6:4 mixture of carbofuran treated and untreated seed was used. Maximum grain yield (45.10 Q/ha) was recorded where treated seed was sown 10 kg/ha and minimum (5.53 Q/ha) in untreated seed sown @ 8 kg/ha.

The data on green fodder yield showed three distinct groups. The first group consisted of carbofuran treated seed sown @ 8 and 10 kg/ha, the second group comprised of the treatment where 6:4 mixture of treated and untreated seed was sown @ 10 kg/ha and the third group, which gave significantly lower yields, consisted of the three treatments in which only untreated seed was sown @ 8, 10 and 12 kg/ha.

The overall results obtained on grain and fodder yields have shown that untreated seed sown @ 8, 10 and 12 kg/ha resulted in significantly lower yields of grain and fodder as compared to treatments where only carbofuran treated seed was used, thus indicating that use of higher seed rate of 12 kg/ha may not serve any useful purpose

under high shoot fly infestation conditions. The carbofuran treated seed used alone or as 6:4 mixture with untreated seed, though showed marginal statistical differences in 1974 trial, were at par in 1975 trial. It is therefore, indicated that use of treated seed @ 10 kg/ha may not be remunerative and the use of 6:4 mixture of treated and untreated seed will effectively control the shoot fly and will reduce the cost of insecticide to the extent of about 40 per cent.

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Year	Sowing date		
	D ₁	D ₂	D ₃
1975	April 25		
1976	April 25	May 23	June 22
1977	May 4	June 2	July 5

Because of excessive rains in 1977, the sowing dates could not be kept the same as in 1976. Randomised block design having four replications except in 1975 (3 replications) with a plot size of 6×8 metres was adopted. The crop was sprayed only against the jassid, *Amrasca biguttula biguttula* (ISHIDA) with dimethoate 30 EC @ 75 ml ai/acre when leaves in the upper canopy showed signs of yellowing and curling along their margins. Such sprays do not affect the bollworm incidence. Four plants per repeat were tagged at random two weeks after the seedling emergence. The flowers formed daily on each test plant were counted and tagged with jewel tags up to the middle of October in each year. The information regarding bollworm attack and rosette formation was also noted. The flowers which turned into harvestable bolls were noted. The shed flowers were collected daily from three quadrates of one-square metre each and dissected for bollworm examination. All the shed flowers without bollworm attack were taken as shed due to physiological factors.

RESULTS AND DISCUSSION

Observations on the pattern of flowering in *F* 414 and *J* 205 sown at different dates and its impact on the bollworm incidence were recorded during 1975 to 1977. The data pertaining to various aspects are discussed below:

Plant age at flowering

It varied from 62.50 ± 12.40 to 91.06 ± 9.065 days in *F* 414 and in *J* 205 from 58.44 ± 8.93 to 91.92 ± 4.60 days under different conditions (Table 1).

Flowers produced per plant:

Total flowers produced per plant varied with year, variety and date of sowing (Table 1). Numbers decreased with the

delay of sowing. It ranged from 27.88 ± 4.54 to 139.44 ± 10.04 in *F* 414 and 22.31 ± 5.27 to 102.69 ± 12.09 in *J* 205 at different sowing dates in different years.

Appearance of 'stress flowers'

The flowers produced during the early period of flowering phase which did not contribute to ultimate yield of seed-cotton were considered as 'stress flowers'. The extent of 'stress flowers' varied with variety, year and date of sowing. Production of these flowers are more in end April (D₁) sown crop as compared with late sown (Table 1).

Flowering period

Both *F* 414 and *J* 295 varieties showed indeterminate type of flowering behaviour. The plants started flowering as early as June and continued till end of crop season (mid-October). Flowering period of the same variety fluctuated greatly at different sowing dates in different years (Table 2). It was longest in end-April (D₁) sown crop and shortest in late-June (D₃) sown crop irrespective of the variety and the year of study.

DASTUR (1950) observed that duration of flowering period was 5 months (June to September) in south Sind and 2.5 months in south west Punjab (Pakistan) in some long duration *hirsutum* varieties. These variations might be due to the difference of varieties and climatic conditions.

Period of maximum flower-formation:

The data presented in Table 2 revealed that maximum flowering occurred in September (Figs. 1 & 2) except in first week of July (D₃) sown crop during 1977 where it occurred during October 1-15 in both the *hirsutum* varieties.

FLOWERING PHASE AND PINK BOLLWORM (*PECTINOPHORA GOSSYPIELLA*) (SAUNDERS) INCIDENCE ON *HIRSUTUM* COTTON

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Observations on the pattern of flowering in *F 414* and *J 205* sown on different dates and its impact on the incidence of the pink bollworm were recorded during 1975 to 1977. Plant age at flowering varied from 60.50 ± 12.40 to 91.06 ± 9.65 days in *F 414* and from 58.44 ± 8.93 to 91.62 ± 4.60 days in *J 205*. Total flowers produced per plant decreased with the delay in sowing. Production of 'stress flowers' was more in end-April sown crop as compared with late sowings. Both *F 414* and *J 205* showed indeterminate type of flowering behaviour. Significantly more flowers were produced in September in both the varieties. However, period of maximum flower transformation into harvestable bolls occurred in August and September. Extent of flower transformation into harvestable bolls varied from 37.47 to 49.81 per cent in *F 414* and 22.36 to 41.14 per cent in *J 205*. All the attacked flowers did not shed. In *F 414* about 9.66 to 30.09 per cent flowers attacked by pink bollworm developed into harvestable bolls against 8.55 to 36.67 per cent in variety *J 205*. Period of maximum pink bollworm attack did not coincide with the periods of maximum flower production and retention. The extent of pink bollworm attack in flowers varied up to 52 per cent in *F 414* and up to 92.3 per cent in *J 205* during different periods. Pink bollworm attack was maximum in the flowers of end-April sown crop and decreased with the delay in sowing. The period of rosette-formation, generally synchronized with the period of pink bollworm attack. It was up to 52 per cent in *F 414* and up to 87.5 per cent in *J 205*. Spotted bollworm incidence was less than that of pink bollworm and was up to 3.3 per cent in *F 414* and up to 9.03 per cent in *J 205*. Maximum flower-shedding was due to physiological factors (other than insects). It was followed by spotted bollworm and pink bollworm respectively.

(Key words: flowering phase, *F 414* and *J 205* cotton, pink bollworm incidence, *Pectinophora gossypiella*)

INTRODUCTION

The pink bollworm (*Pectinophora gossypiella* (SAUNDERS)) is a limiting factor in cotton cultivation in the state. Most of the ecological studies on this pest have been made without considering the phenology of crop. These studies are not only required for chemical control considerations but also for making important decisions in overall pest management. The information available on the crop phenology in relation to bollworm damage to cotton is scanty in India in general and in the Punjab in particular. KATIYAR (1977) studied the impact of sequence of flowering in some *hirsutum*

varieties and bollworm damage from August onward at New Delhi. The information regarding complete flowering phase under different sowing conditions in relation to bollworm incidence is still lacking. The present studies were undertaken on pink bollworm infestation in relation to the phenology of cotton crop. A part of this pertaining to flowering phase is reported in this paper.

MATERIALS AND METHODS

These studies were carried out at Ludhiana during 1975 through 1977 on *hirsutum* varieties *F 414* and *J 205*. The sowing dates were as under:

- KEM, T.R. (1977) Selection of a strain of *Tribolium castaneum* (HERBST) resistant to phosphine. *J. ent. Res.*, **1**(2) : 213-217.
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of phosphine to stored product insects in laboratory investigations, which have been discussed by various workers (HOWE, 1974; BANKS, 1974; BOND *et al.*, 1969). It has been reported that phosphine concentrations are a critical factor, as 0.5 mg/l has been shown to be the upper concentration limit of linear dosage-mortality relationship at 25°C. Further, the insects are reported to become narcotised which to some extent protects them from the lethal effects of the fumigant if the exposure periods are short and the concentrations high (CHAMP & DYTE, 1976). In the present selection programme the first factor would appear to have played some role as from 13th generation onward the concentration (0.4 mg/l) nearly approached the stated upper limit and its application probably failed to intensify the selection and hence appreciable increase in resistance was not obtained thereafter, the resistance ratio enhanced from 5.8 in the 12th to 5.9 in the 17th generation. However, as far as the tendency of the adults to become narcotised was concerned it was not observed and the exposure period (24 hr) used in the investigations was sufficient to produce high percentage mortality during selection till the last generation. In the later generations another serious difficulty, namely, the reduction in the progeny emergence was experienced which hampered the selection programme. Experiments showed that this reduction in adult progeny was due to lower fecundity of the resistant females as well as the adverse effect of their treatment with phosphine. The resistant strain laid 24% less eggs than the susceptible females. Progeny reduction in phosphine-resistant ($\times 6.8$) *T. castaneum* has also been reported by WINKS (1971). So far as reduced fecundity of phosphine treated females is concerned, the results will be reported later (SAXENA, 1979).

Phosphine resistance was observed in immature stages also and the degree of

resistance was egg $\times 2.9$, 1st instar larva $\times 4.4$, last instar larva $\times 3.3$ and pupa $\times 3.5$. There have been few investigations on the occurrence of resistance in immature stages. It was reported that when adults of *T. castaneum* were used in selection for resistance to phosphine, some resistance might occur in pupal stage (ANON., 1974). In *S. oryzae* some resistance in immature stages did develop to methyl bromide when adults were selected (MONRO *et al.*, 1972; UPITIS *et al.*, 1973). Thus the present studies give definite evidence of development of resistance in the immature stages consequent upon selection with adults although the degree of resistance in these stages was lower than that in the adults. The development of resistance in immature stages has important practical implications. Such resistant strains with their immature stages also showing resistance would be difficult to kill and hence pose a problem in the control programmes.

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TABLE 3. Comparative biology of the susceptible and phosphine-resistant strains of *T. castaneum*.

Strains	Period (days)			Oviposition female (70 days)
	Incubation	Larval	Pupal	
Susceptible	4.3 ± 0.18	15.2 ± 0.28	7.0 ± 0.09	300.5 ± 14.73
Resistant	3.7 ± 0.14	15.4 ± 0.19	6.7 ± 0.11	228.0 ± 8.43

TABLE 4. Toxicity data of phosphine to the immature stages of susceptible (S) and phosphine-resistant (R) strains of *T. castaneum*.

Stage	Strain	Heterogeneity d.f. × 2	Regression Coefficient	LC ₅₀ (mg/l)	Feducial limits	Resistance ratio R/S
Egg	S	5 2.456	5.368 ± 0.71	0.160	0.138-0.208	2.9
	R	5 1.581	3.510 ± 0.94	0.471	0.367-0.604	
First instar	S	4 1.464	1.171 ± 0.08	0.013	0.008-0.021	4.4
	R	4 4.746	5.920 ± 1.51	0.057	0.051-0.064	
Last instar	S	4 2.240	2.136 ± 0.12	0.027	0.020-0.037	3.3
	R	4 0.854	3.062 ± 0.66	0.090	0.070-0.115	
Pupal	S	4 2.403	3.535 ± 0.46	0.092	0.071-0.117	3.5
	R	4 6.090	7.212 ± 1.41	0.322	0.279-0.372	

phosphine among the various developmental stages for both the strains viz., phosphine-resistant and susceptible, was same i.e., 1st instar larva > last instar larva > pupa > egg.

DISCUSSION

It was thus seen that resistance to phosphine developed in *T. castaneum* as a result of selection of adults in successive generations. After 16 selections the adults were 5.9 times resistant to phosphine as compared to the parental susceptible strain. It was not possible to increase the resistance further with the present selection pressure.

There have been few attempts to select phosphine-resistant strains in the laboratory,

and the results obtained in the present studies are in conformity with those reported earlier. In *T. castaneum* a resistance level of × 5 in adults was reported by WINKS (1969) in a previously lindane-resistant strain and the level increased to × 6.5 in F₁ progeny. WINKS (1971) also reported an × 6.8 increase in resistance in a strain after 6 selections. KEM (1975) selected a strain with 11.9 resistance in 10 generations. In the case of *S. oryzae*, MONAO *et al.* (1972) developed a strain 3 times resistant to phosphine in 41 generations after 28 selections.

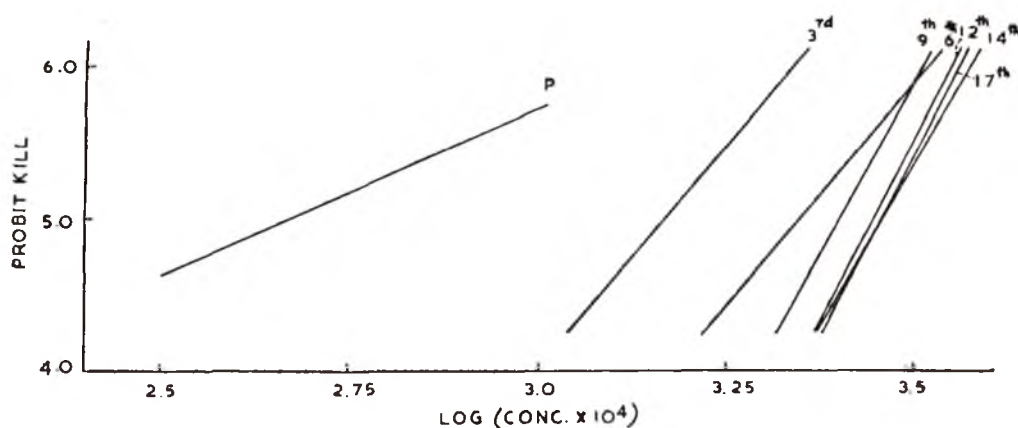
There are a number of practical difficulties in selection for resistance to phosphine in *T. castaneum* due to the problems of toxicity

TABLE 1. Doses of phosphine used in successive generations of *T. castaneum* during the selection programme.

Generation	dose (mg/l)	Generation	dose (mg/l)	Generation	dose (mg/l)
1st (Parental)	0.08	6th	0.2	11th	0.3
2nd	0.1	7th	0.25	12th	0.3
3rd	0.1	8th	0.25	13th	0.4
4th	0.2	9th	0.25	14th	0.4
5th	0.2	10th	0.3	15th	0.4
				16th	0.4

TABLE 2. Toxicity data of phosphine to the adults of the different generations of *T. castaneum*, during selection for phosphine resistance.

Generation	Heterogeneity d.f. $\times 2$	Regression Coefficient	LC ₅₀ (mg/l)	Feducial limits	Resistance Ratio
Parent	4 2.080	2.153 ± 0.32	0.048	0.035-0.066	..
3rd	4 5.634	5.829 ± 0.27	0.149	0.118-0.171	3.1
6th	3 8.668	6.054 ± 0.53	0.226	0.195-0.261	4.7
9th	4 1.992	9.135 ± 0.56	0.255	0.220-0.294	5.3
12th	4 3.779	9.659 ± 1.20	0.279	0.242-0.324	5.8
14th	4 7.300	8.417 ± 1.06	0.284	0.245-0.328	5.9
17th	4 5.180	9.418 ± 1.58	0.281	0.243-0.325	5.9

Fig. Log dosage-probit mortality regression lines for phosphine to the adults of *T. castaneum* for different generations of selection.

required doses of air-fumigant mixture were introduced into the fumigation flask, and each dose was replicated thrice. The insects were exposed for 24hr and then removed to specimen tubes (7×2.5 cm) each containing a folded paper strip. Insects were allowed 48 hr to recover after which the mortality was recorded.

The method of fumigation for immature stages was the same as followed for adults. Each stage viz., egg, 1st instar larva, last instar larva and pupa was tested separately. After the exposure period of 24hr, the eggs were observed for hatchability, larvae were transferred to sterilized flour and observed till pupation, and pupae were observed for adult emergence. The mortality in respective cases was based on unhatched eggs, unpupated larvae and unemerged pupae. The mortality data were subjected to probit analysis (FINNEY, 1952).

Selection for resistance:

The selection was initiated with a laboratory culture of *T. castaneum*, which had no history of exposure to phosphine. The selection was carried out in adult stage. Since the tests* showed that both the sexes were equally susceptible to phosphine, mixed population was used for selection. The selection pressure was applied in each successive generation starting with the parent culture. The fumigant dose was selected in such a way that the treatment with it could result in mortality above 60% preferably between 60 to 80%. In order to achieve this, the doses were increased at intervals determined on the basis of bioassay tests in certain generation. The doses applied in each generation and the number of generations of selection are given in Table 1. For every selection about 3000 to 4000 adults (8 to 10 days) old were used. Fifty insects were taken in each fumigation flask and after exposure to the fumigant for 24 hr, the insects were removed and allowed to recover for 48 hr. The survivors were transferred to flour for oviposition and the next generation was reared. The culture of the selected phosphine-resistant insects was maintained similar to that mentioned earlier. The parental culture was also maintained simultaneously without any selection pressure as the susceptible strain. The increase in resistance was determined on the basis of bioassay tests and the resistance ratio was worked out by dividing the LC_{50} value of phosphine to the selected strain by that to the susceptible.

* LC_{50} value of phosphine to males and females was found to be 0.055 and 0.048 respectively. The values are statistically similar.

RESULTS

The results (Table 2) show that there was an increase in resistance to phosphine as a result of selection for 16 generations as the LC_{50} value increased from 0.048 mg/l in the parental to 0.281 in the 17th generation and the resistant strain thus selected was 5.9 times resistant to phosphine. It may be seen that during selection the increase in resistance was progressive and marked only till the 12th generation and subsequent selections did not bring about significant increase in spite of higher selection dosages. The resistance ratio were 3.1, 4.7, 5.3, 5.8, 5.9 and 5.9 in the 3rd, 6th, 9th, 12th, 14th and 17th generations respectively. This is also confirmed from comparison of the dosage-mortality regression lines (Fig.1) which moved to the right and became steeper in successive generations of selection upto the 12th generation only, thereafter, the increase in resistance was not much as the lines did not show any significant shift in position or slope.

The comparison of the biology (Table 3) of the phosphine-resistant and susceptible strains shows that there is no significant difference between the duration of the developmental stages of the two strains. However, there was a significant reduction in the fecundity of the resistant strain and it laid 24% less eggs as compared to the susceptible strain.

The results on the susceptibility of immature stages to phosphine (Table 4) show that LC_{50} values for all the stages tested were higher for the selected strain than the susceptible, and all the stages were, therefore, resistant to the fumigant. The resistance ratios for egg, 1st instar larva, last instar larva and pupa of the selected strain were 2.9, 4.4, 3.3, and 3.5 respectively.

The relative order of susceptibility to

LABORATORY SELECTION OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST) FOR RESISTANCE TO PHOSPHINE

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A phosphine-resistant strain of *T. castaneum* was developed in the laboratory by selecting the adults in successive generations and after 16 generations the adults were 5.9 times resistant to phosphine as compared to the parental strain. The immature stages of the selected strain also showed resistance to phosphine, and their degree of resistance was: egg $\times 2.9$, 1st instar larva $\times 4.4$, last instar larva $\times 3.3$, and pupa $\times 3.5$. The phosphine-resistant females had 24% lower fecundity as compared to the susceptible while there was no difference in the duration of the developmental stages of the two strains.

(Key words: phosphine resistant strain, *Tribolium castaneum*, laboratory selection)

INTRODUCTION

The development of resistance to fumigants in stored product pests is emerging lately as a serious problem under practical conditions. The FAO global survey report (CHAMP & DYTE, 1976) shows that fumigant resistant strains are now prevalent in several countries. Out of the 894 strains of various species tested, 42 showed increase in tolerance to methyl bromide and 82 out of 849 showed increase in tolerance to phosphine. Although no appreciable resistance to methyl bromide could be detected in strains from India, resistance to phosphine was shown to be present in strains of 3 species, viz., *Sitophilus oryzae* (LINNAEUS), *Tribolium castaneum* (HERBST) and *Rhyzopertha dominica* FABRICIUS. In *R. dominica* the resistance to phosphine was reported to be as high as 7.2 times. However, phosphine resistance levels in field strain were low in the case of *T. castaneum*.

In view of the growing importance of fumigant resistance in stored product pests the present studies were undertaken. The paper deals with selection for resistance to phosphine in *T. castaneum*.

MATERIAL AND METHODS

Rearing of the test insect: The culture of *T. castaneum* was maintained by rearing it on sterilized wheat flour containing 5% Brewer's yeast. 100 g of flour was put in a rearing jar measuring 15 \times 10 cm. About 300-400 insects were released in the jar for oviposition, and after 4-5 days they were sieved out and released in another jar containing flour. A succession of such jars was maintained at $30^{\circ} \pm 2^{\circ}\text{C}$ in order to get a regular supply of insects of known age.

Preparation of fumigant:

Phosphine gas was generated by the decomposition of aluminium phosphide pellets, obtained from M/s Excel Industries Ltd. The air-fumigant mixture was prepared in 10 litre flask fitted with ground glass stopper, by putting a pellet weighing 0.6 gm, which on decomposition gave 200 mg of phosphine gas. The required doses of air-fumigant mixture were calculated and transferred in fumigation flasks (250 ml) by using the method described by PRADHAN & COVINDAN (1953) with some minor modifications necessary for accurate transfer of air-fumigant mixture.

Method of bioassay:

The adults (8 to 10 days old) were counted and introduced directly into the fumigation flasks. A paper strip with many folds was placed in the flask for insects to rest upon. The flasks were then closed with one way ground glass stopper. The

former two were better than the latter which in turn was better than the remaining insecticide. The maximum mortality was again in endosulfan in larvae feeding on 7 days old spray deposits followed by quinalphos and monocrotophos. The least effective insecticide was fenitrothion.

(2) *Effect of insecticidal spray-deposits on mortality of newly hatched larvae:*

There was seventy per cent mortality of newly-hatched larvae within 72 hours which were fed on cotton leaves having 2-day-old spray deposits of monocrotophos quinalphos and endosulfan at 0.5 kg ai/ha and these were significantly superior to the remaining treatments. But when larvae were fed on 4 days old-spray-deposits of monocrotophos quinalphos and endosulfan 0.3 kg ai/ha mortality was as good as in higher dosages of 0.5 ai/ha and all were better than carbaryl and fenitrothion. The mortality of newly-hatched larvae on 7 days old-spray-deposits of endosulfan 0.5 kg was maximum (64.5%) followed by quinalphos 0.5 kg (56.1%).

The least effective treatments were carbaryl and fenitrothion.

No information is available on effectiveness of quinalphos, monocrotophos, phenthoate and phosalone. In laboratory studies endosulfan was found to be effective against this pest by PRADHAN *et al.* (1960) and TRIPATHI (1966). In field trial BAKHETIA & SIDHU (1971) also reported this insecticide to be the most effective. In the present studies also this insecticide was most effective.

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experiment it is clear that even lower dosage (0.3 kg ai/ha) of monocrotophos and quinalphos were superior to higher dosage of phosalone and phenthoate (0.9 kg ai/ha) and carbaryl (1.25 kg ai/ha) but there was no difference between the former and fenitrothion at 0.75 kg and endosulfan 0.3 kg. The latter two were at par with the remaining insecticides. The data of the third experiment show that higher dosages of the promising insecticides i.e., monocrotophos, quinalphos and endosulfan at 0.9 kg ai/ha resulted in 61–69% mortality of the young larvae migrating from treated fields. Fenitrothion 0.75 and carbaryl 1.50 and 2.0 kg caused only 33 to 41% mortality of such larvae. Thus only monocrotophos, endosulfan and quinalphos at 0.9 kg ai/ha were found effective against such larvae.

(c) Untreated larvae feeding on treated leaves (larvae migrating into treated fields):

In the first experiment the maximum mortality of such larvae where they migrated two days after spray was 71.2% in quinalphos 0.75 kg and minimum 29.7% in phenthoate 0.5 kg (Table 1). There was no

difference between quinalphos monocrotophos and endosulfan at 0.75 kg and all were better than phenthoate, phosalone and quinalphos. Similar trend was observed in larvae migrating 4 days after spraying. The maximum mortality (37.8%) of larvae migrating 7 days after spraying was in quinalphos 0.75 kg and minimum (0.8%) in phosalone 0.5 kg. There was no difference between monocrotophos, quinalphos, endosulfan and fenitrothion 0.75 kg and all these were better than the remaining insecticides. In the second experiment, 2 days after spraying the mortality varied from 21.9 to 39.7%. There was no difference between monocrotophos, quinalphos, endosulfan at 0.3 kg fenitrothion 0.75 kg and phosalone 0.9 kg ai/ha. Similar trend was in 4 days old spray deposits and there was no difference in 7 days old spray deposits. In the third experiment where higher dosages of all the promising insecticides were tested the maximum mortality of such larvae was 88.6% in quinalphos and the minimum 44.5% in fenitrothion both at 0.9 kg ai/ha. There was no difference between quinalphos, endosulfan and monocrotophos. But 4 days after spraying the

TABLE 2. Effect of insecticidal spray-deposits on newly-hatched Bihar hairy caterpillar.

Insecticide kg ai ha		Mortality (%) of newly hatched larvae after 72 hrs		
		2-day-old-deposit	4-days-old-deposit	7-days-old-deposit
Monocrotophos	0.30	82.1 (64.96)	77.8 (61.88)	48.9 (44.37)
	0.50	100.0 (90.00)	81.6 (64.59)	48.9 (44.37)
Quinalphos	0.30	76.7 (61.14)	69.0 (55.14)	46.7 (43.08)
	0.50	100.0 (90.00)	75.0 (60.00)	56.1 (49.48)
Endosulfan	0.30	94.3 (74.76)	69.5 (56.50)	53.5 (48.99)
	0.50	100.0 (90.00)	75.99 (60.00)	64.5 (53.43)
Fenitrothion	0.75	63.00 (52.55)	41.8 (43.15)	28.9 (32.49)
Carbaryl	1.25	64.5 (53.43)	50.8 (45.45)	31.0 (33.82)
CD (p=0.05)		(12.97)	(9.41)	(5.88)

Figures in parentheses are angular transformations.

Table 1 (Contd.)

1	2	3	4	5	6	7	
Phosalone	0.50	57.8(49.51)	24.4(29.59)	16.8(24.22)	26.7(31.11)	24.4(29.59)	19.7
Fenitrothion	0.75	66.8(54.81)	35.4(36.52)	22.7(29.45)	39.7(39.08)	24.4(29.59)	10.9
Endosulfan	0.30	72.3(58.24)	26.7(31.11)	39.7(26.35)	29.9(33.15)	28.9(32.40)	17.4
Carbaryl	1.25	65.3(53.88)	19.7(26.32)	19.7(26.35)	26.4(30.93)	24.4(29.89)	10.9
CD (p = 0.05)		(9.33)	(7.17)	(10.02)	(6.81)	(4.27)	NS
<i>Experiment 3</i>							
Monocrotophos	0.90 *		48.9(44.37)	60.9(51.29)	71.8(57.88)	64.5(53.43)	55.5(48.18)
Quinalphos	0.90 *		72.3(58.25)	69.0(56.18)	80.6(70.77)	78.1(62.06)	64.5(53.43)
Fenitrothion	0.90 *		36.6(37.90)	40.6(39.70)	44.5(41.82)	33.2(35.19)	28.7(32.49)
Endosulfan	0.90 *		75.8(60.00)	60.0(51.75)	60.0(75.00)	83.7(66.19)	67.5(55.26)
Carbaryl	1.50 *		28.8(32.49)	33.2(35.19)	52.10(62.3)	48.9(44.37)	51.2(45.67)
	2.00 *		37.7(37.90)	35.3(36.32)	66.7(54.76)	47.9(44.33)	40.9(39.23)
CD (p = 0.05)			(4.28)	(15.17)	(14.35)	(6.66)	(6.48)

Figures in parentheses are angular transformation.

*Not tested.

TABLE 1. Effectiveness of different insecticides against Bihar hairy caterpillar.

Insecticide kg ai/ha	Corrected mortality (%) after 72 hours					
	Treated larvae and treated food		Treated larvae and untreated food		Untreated larvae (IV-V stage) and treated food (days after spray)	
	I-II Stage	IV-V Stage	IV-V Stage	2	4	7
<i>Experiment 1</i>						
Monocrotophos	0.50	93.6(77.83)	26.5(30.97)	46.6(43.08)	62.4(52.18)	46.7(43.11)
	0.75	100.0(90.00)	48.9(44.37)	57.7(49.44)	66.7(54.75)	53.4(46.92)
Quinalphos	0.50	91.3(72.79)	44.5(41.82)	51.2(45.66)	57.0(49.55)	44.5(41.82)
	0.75	100.0(90.00)	46.7(43.08)	62.3(52.14)	71.2(57.51)	51.1(45.64)
Phenthoate	0.50	31.0(32.82)	4.4(12.10)	8.7(17.13)	19.7(26.35)	15.4(22.12)
	0.75	41.7(40.19)	10.9(19.26)	13.3(21.39)	22.2(28.08)	15.4(23.12)
Phosalone	0.50	35.4(36.49)	10.5(18.89)	10.9(19.25)	22.2(28.08)	13.3(21.39)
	0.75	53.5(46.99)	37.7(24.84)	17.4(25.63)	28.9(32.49)	10.9(19.25)
Fenitrothion	0.50	66.8(54.81)	15.4(28.11)	19.7(26.33)	55.7(48.25)	32.6(34.82)
	0.75	97.0(88.00)	58.5(49.88)	53.5(48.18)	64.6(53.48)	51.1(45.66)
Endosulfan	0.75	100.0(90.00)	67.4(55.18)	66.7(54.76)	33.1(35.11)	18.7(25.65)
	1.00	70.2(56.92)	17.7(24.63)	22.2(28.08)	26.9(32.49)	10.9(19.25)
Carbaryl						8.7(17.13)
CD (p = 0.05)		(12.20)	(10.14)	(5.61)	(7.66)	(4.96)
						(11.77)
<i>Experiment 2</i>						
Monocrotophos	0.30	76.7(61.12)	10.9(19.25)	35.4(36.57)	33.2(35.19)	24.2(29.45)
Quinalphos	0.30	79.2(62.87)	26.5(30.97)	35.4(36.49)	37.6(37.90)	30.5(32.49)
Phenthoate	0.90	48.9(44.37)	24.9(22.71)	14.9(22.71)	21.9(27.87)	17.4(24.63)
						17.7

in first experiment their dosages were decreased to 0.3 kg ai/ha. Phenthoate and phosalone at 0.75 kg were not effective, so they were tested at 0.9 kg ai/ha. The dosages of fenitrothion and carbaryl were increased to 0.75 and 1.25 kg ai/ha as the lower dosages failed to give good control of young larvae. The information on their effectiveness against mature larvae was also studied. The crop was sprayed in the first week of September. The treatments were replicated three times in randomized layout having plot size of 20×10 m. All the observations were recorded as in the previous experiment.

The third experiment was done to study the effectiveness of higher dosages of promising insecticides against full grown larvae. Monocrotophos, quinalphos, fenitrothion and endosulfan each at 0.90 kg and carbaryl at 1.5 and 2.0 kg ai/ha were tested. The treatments were replicated thrice in randomized block design with plot size of 15×10 m. The spray was given in middle of September with knapsack pump. The observations on mortality of full-grown larvae feeding on treated food was recorded in the same way as in first experiment. In addition, mortality of treated young larvae feeding on untreated food and untreated larvae feeding on treated food having 2, 4 and 7 days old spray-deposits was also determined as in first experiment.

Fourth experiment was done in the laboratory where the mortality of newly hatched larvae was determined on cotton leaves having, 2, 4 and 7 days old spray-deposits. Monocrotophos, quinalphos and endosulfan at 0.3 and 0.5 kg, fenitrothion 0.75 kg and carbaryl at 1.25 kg ai/ha were evaluated. Three batches of fifteen larvae each were reared on cotton leaves with different spray deposits. The mortality counts were taken after 72 hrs.

RESULTS AND DISCUSSION

The data on the effectiveness of various insecticides against Bihar hairy caterpillar are given in Table I and are discussed below.

(a) *Treated larvae feeding untreated food (i.e. non-migrating larvae):*

In the first experiment monocrotophos, quinalphos and endosulfan were significantly better than the other insecticides against young larvae causing 91–100% mortality. This was followed by carbaryl and fenitrothion. Phosalone and phenthoate were less

effective. Endosulfan at 0.5 kg and quinalphos and monocrotophos at 0.75 kg ai/ha were most effective. Endosulfan at 0.5 kg and quinalphos and monocrotophos at 0.75 kg ai/ha were also most effective against mature larvae causing 48.9 to 67.4% mortality. The least effective insecticide was phenthoate 0.5 kg where only 4.4% larvae died. In the second experiment the lower dosage (0.3 kg ai/ha) of monocrotophos and quinalphos was significantly better than phenthoate and phosalone and at 0.9 kg ai/ha against young larvae. But against mature larvae there was no difference between fenitrothion 0.75 kg, endosulfan 0.3 kg, phosalone 0.90 kg and quinalphos 0.3 kg and all were better than monocrotophos 0.3 kg. In the third experiment were tested for control of mature larvae, the differences among various insecticides were significant. Quinalphos and endosulfan at 0.9 kg ai/ha were significantly superior to the other insecticides. These were followed by monocrotophos 0.9 kg which was significantly better than fenitrothion 0.90 kg and carbaryl 1.50 and 2.0 kg ai/ha. Carbaryl 1.50 kg was significantly inferior to all other insecticides. So endosulfan, monocrotophos and quinalphos at 0.3 kg ai/ha were the most effective against non-migrating young larval population, but against mature larvae only quinalphos and endosulfan at 0.90 kg ai/ha were effective.

(b) *Treated larvae feeding on untreated food (larvae migrating out of the treated feeds):*

These observations were made only on the old larvae. In the first experiment the differences were significant and per cent mortality varied from (8.7 phenthoate 0.5 kg) to 66.7 (endosulfan 0.75 kg). There was no difference between endosulfan, quinalphos and monocrotophos at 0.75 (kg ai/ha but these were significantly better than the remaining insecticides. From the second

CHEMICAL CONTROL OF BIHAR HAIRY CATERPILLAR INFESTING COTTON

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Cotton crop was severely attacked by Bihar hairy caterpillar (*Diacrisia obliqua* WALKER) during 1977. Three field experiments were conducted where four new insecticides i.e., quinalphos, monocrotophos, phenthoate and phosalone were compared with endosulfan, fenitrothion all at 0.3, 0.5, 0.75 and 0.9 kg ai/ha and carbaryl at 1.0, 1.25, 1.50 and 2.0 kg ai/ha. Monocrotophos, quinalphos and endosulfan at 0.3 kg were effective against young larvae. phosalone and phenthoate were least effective. But against mature larvae, the mortality varied from 60–69% with higher dosages of 0.9 kg ai/ha of monocrotophos, quinalphos and endosulfan. Fenitrothion and carbaryl gave the moderate control of young and mature larvae. Monocrotophos, quinalphos and endosulfan 0.5 kg ai/ha were most effective against newly-hatched larvae of this pest.

(Keywords: Bihar hairy caterpillar, *Diacrisia obliqua*, chemical control)

INTRODUCTION

The Bihar hairy caterpillar (*Diacrisia obliqua* WALKER) is a sporadic pest in the Punjab. It occurred in epizootic form on *kharif* crops during 1977. Severe attack of this pest was observed in the cotton fields especially where sesame (*Sesamum orientalis* L.) was grown in cotton crop along the plot periphery for getting additional benefits. The pest after defoliating these plants attacked the cotton crop and defoliated it within two to three days. Survey conducted revealed the skeletonized plants with stem, branches and leaf-veins. A number of cotton fields free from sesame were also badly attacked by this pest. The population was so high that weeds and trees like *kikar* (*Acacia arabica*) were heavily infested by this pest. So some experiments were conducted for the control of this pest on the cotton crop and results of this are given in this paper.

MATERIALS AND METHODS

The insecticides which are recommended for the control of cotton pests in the Punjab (PAU, 1977) and new insecticides were screened against the 1st

and 2nd instar stage and full grown larvae of this pest. Effectiveness of these insecticides was studied under three conditions, viz. (i) treated larvae feeding on treated food; (ii) treated larvae feeding on untreated food; and (iii) untreated larvae feeding on treated food having 2, 4 and 7 days old spray deposits.

In the first experiment endosulfan 0.50 and 0.75 kg, fenitrothion 0.5 kg and carbaryl 1.0 kg ai/ha as recommended for control of cotton pests were tested along with monocrotophos (Nuvacon 40), quinalphos (Ekalux 25 EC), phenthoate (Phendal 50 EC), and phosalone (Zolone 35 EC) each at 0.5 and 0.75 kg ai/ha. The crop was sprayed in end August with the knap-sack pump. The different treatments were in randomized layout with three replications. The plot size was 15 × 10 m. Large number of young I and II stage and full grown larvae (IV and V stage) were collected from the middle of each plot within an hour of spraying. One set of fifteen larvae each of the young and mature larvae from every plot was reared on treated leaves of same insecticides and another batch of fifteen larvae was fed on untreated leaves. A large number of larvae were collected from untreated fields and were fed on treated leaves having 2, 4 and 7 days old spray-deposits. Each treatment was having three replications of fifteen larvae. The mortality of larvae was recorded after 72 hrs.

Since monocrotophos, quinalphos and endosulfan proved effective at 0.5 kg ai/ha against young larvae

TABLE 1. Flowering potential of *hirsutum* varieties F 414 and J 205 under natural bollworm infestation during different years.

Sowing date	F 414			J 205		
	1975*	1976	1977	1975	1976	1977
		<i>Plant age at flowering (days)**</i>				
D ₁		62.50 ± 12.40	71.69 ± 21.58	+	58.44 ± 8.93	71.69 ± 21.91
D ₂	—	71.13 ± 12.29	91.06 ± 9.65	—	75.62 ± 11.61	91.62 ± 4.60
D ₃	—	65.25 ± 4.78	66.19 ± 6.99	—	65.62 ± 6.67	65.00 ± 5.41
		<i>Number of flowers produced per plant**</i>				
D ₁	65.78 ± 9.12	139.44 ± 10.04	60.69 ± 3.72	67.67 ± 8.48	102.69 ± 12.09	46.13 ± 11.25
D ₂	—	57.69 ± 17.64	27.88 ± 4.54	—	63.62 ± 10.75	26.81 ± 7.95
D ₃	—	48.44 ± 17.15	35.63 ± 6.65	—	52.56 ± 5.91	22.31 ± 5.27
		<i>Percentage of stress flowers**</i>				
D ₁	0.49 (up to July 23)	4.22 (up to July 7)	1.05 (upto July 31)	7.06 (upto July 31)	7.39 (Up to July 15)	4.95 (up to July 23)
D ₂	—	5.60 (up to July 23)	0	—	0.87 up to July 31)	0
D ₃	—	0	0	—	0.81 (up to Aug 23)	0

*Observations were started from 1st July.

**Mean of 3 replications in 1975 and 4 replications during 1976 and 1977.

+not studied in 1975.

TABLE 2. Flower formation behaviour of *hirsutum* varieties F 414 and J 205 in different years.

Sowing dates	F 414			J 205		
	1975	1976	1977	1975	1976	1977
	<i>Flowering period 1</i>					
D ₁	July 16-Oct. 15	June 8-Oct. 7 (122)	June 8-Oct. 15 (114)	July 8-Oct. 15	June 8-Oct. 7 (122)	June 24-Oct. 15 (114)
D ₂	—	July 16-Oct. 7 (84)	Aug. 8-Oct. 15 (69)	—	July 16-Oct. 7 (84)	Aug. 8-Oct. 15 (69)
D ₈	—	Aug. 24-Oct. 7 (45)	Sept. 1-Oct. 15 (45)	—	Aug. 16-Oct. 7 (53)	Sept. 1, Oct. 15 (45)
	<i>Period of maximum flower-formation</i>					
D ₁	Aug. 16-Sept. 15 (67.20)	Sept. 1-15 (38.05)	Sept. 1-7 (33.70)	Sept. 16-23 (19.40)	Sept. 8-15 (25.40)	Sept. 8-23 (29.05)
D ₂	—	Sept. 1-15 (48.20)	Sept. 8-23 (39.60)	—	Sept. 8-15 (36.60)	Sept. 8-23 (51.60)
D ₈	—	Sept. 8-15 (57.80)	Oct. 1-15 (31.00)	—	Sept. 8-23 (69.90)	Oct. 1-15 (56.00)
	<i>Period of maximum flower transformation into harvestable bolls</i>					
D ₁	Aug. 1-Sept. 23	Aug. 16-Sept. 7	Aug. 1-23 Oct. 1-7	Aug. 16-Sept. 30 Oct. 8-15	Aug. 24-Sept. 15	Aug. 1-31
D ₂	—	Aug. 8-31	Oct. 1-15	—	Aug. 16-31	Sept. 16-23
D ₈	—	Aug. 24-Sept. 23	Sept. 24-30	—	Aug. 24-Sept. 15	Oct. 1-7

1 Figures in parentheses indicate the days for flowering period.

2 Figures in parentheses indicate per cent flowers formed during the given period.

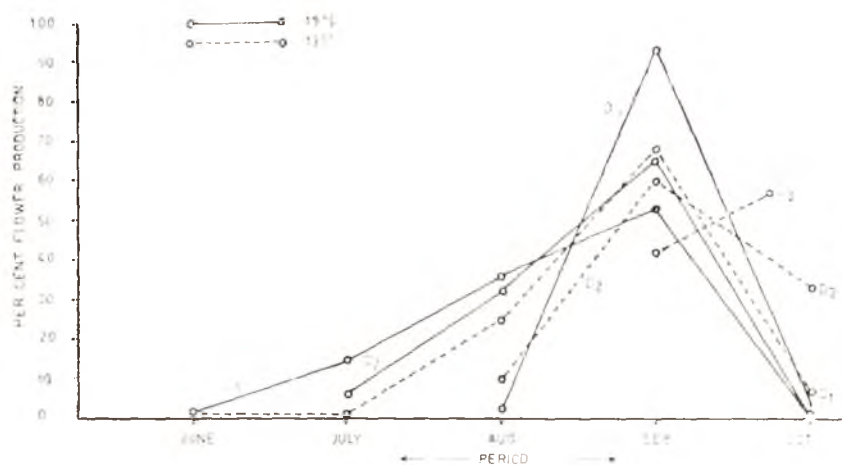


Fig. 1. Pattern of flower production in *F 414* at different sowing dates.

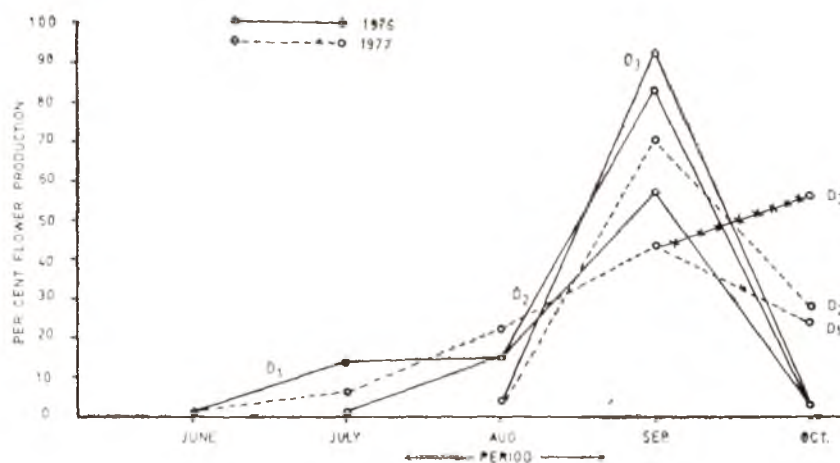


Fig. 2. Pattern of flower production in *J 205* at different sowing dates.

Normal sown *J 34* produced maximum flowers during August at Ludhiana (SANDHU, 1974) and *H 777* and *Bikaneri Narma* from last week of August to last week of September at Hissar (ANONYMOUS, 1978). However, they did not study different dates of sowing.

Period of maximum flower transformation into harvestable bolls:

It differed from that of maximum flower production both the varieties. Also it varied for various dates of sowing in different

year (Table 2). In general, it occurred in August and September in both the varieties at different sowing dates except during 1977 (also in *J* 205 during 1975), when it occurred even in October, in variety *F* 414. It might be due to exceptionally high rains during this year, which was 13.97 mm in 1977, 1.05 mm in 1976 and 6.59 mm in 1975.

KATIYAR (1977) observed that 60 per cent flowers were set as bolls during mid-August to mid-September in normal sown *hirsutum* varieties *PS* 10, *H* 14, *SS* 265, *Badnawari*-1, 9/11 and *Sanguineum* at New Delhi. The present studies also showed a similar trend with minor variations which may be attributed to prevailing environmental conditions and the variety.

Extent of flower transformation into harvestable bolls:

The percentage of flower transformation into harvestable bolls was recorded higher in variety *F* 414 than in *J* 205 except at D_3 during 1977 (Table 3). It also increased with the delay in sowing of both the varieties during 1976 to 1977 with the exception of D_3 (1977) in *F* 414. The average of 1975 to 1977 for end-April (D_1) sown crop is depicted in Fig. 3. It was 39.2 per cent in variety *F* 414 and 29.5 per cent in variety *J* 205.

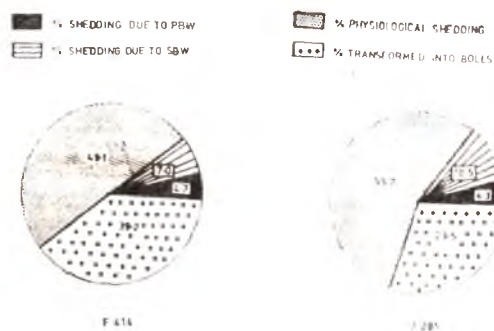


Fig. 3. Pattern of shedding of flowers in *F* 414 and *J* 205 :

It was found that all the flowers attacked by pink bollworm did not shed. In *F* 414 about 9.66 to 30.09 per cent flowers developed into harvestable bolls against 8.55 to 36.67 per cent in variety *J* 205. Further, four out of 24 flowers in *J* 205 developed into harvestable bolls during the course of study. These findings are contrary to the observations of BURT (1916) who reported that all the pink bollworm attacked flowers failed to set as bolls at Allahabad (U.P.)

Pink bollworm incidence :

The incidence of bollworm was recorded in terms of the number of damaged and rosetted flowers separately and the data is given in Tables 4 and 5 and Figs. 5 and 6.

Period of pink bollworm attack :

The pink bollworm attack to the flowers varied from last week of June to October 15 in both the varieties during different years.

Period of maximum pink bollworm attack :

Generally it did not coincide with the period of maximum flower production and retention as harvestable bolls.

Per cent pink bollworm attack :

Variety *F* 414 always suffered lesser pink bollworm infestation than *J* 205. The extent of pink bollworm attack in flowers varied up to 52 per cent in *F* 414 and up to 92.3 per cent in *J* 205 during different periods of flowering phase.

Pink bollworm attack was maximum in the flowers of end-April sown crop (D_1). It started decreasing with the delay in sowing and was minimum in end-June or first week of July (D_5) sown crop.

TABLE 3. Per cent flowers transformed into harvestable bolls.

Sowing date	F 414			J 205		
	1975	1976	1977	1975	1976	1977
				<i>Flowers transformed into harvestable bolls*</i>		
D ₁	38.34	37.47	41.92	29.64	36.46	22.36
D ₂	—	40.85	42.60	—	31.34	33.80
D ₃	—	49.81	35.79	—	41.14	38.38
				<i>Pink bollworms attacked flowers transformed at harvestable bolls</i>		
D ₁	20.00	30.09	9.66	21.99	22.62	8.55
D ₂	—	29.27	23.33	—	36.67	18.40
D ₃	—	26.92	14.21	—	16.67	18.85

*Mean of 3 replications of 1975 and 4 replications in 1976 and 1977.

TABLE 4. Pattern of bollworm attack on the flowers of *hirsutum* varieties F 414 and J 205 during the flowering phase in different years.

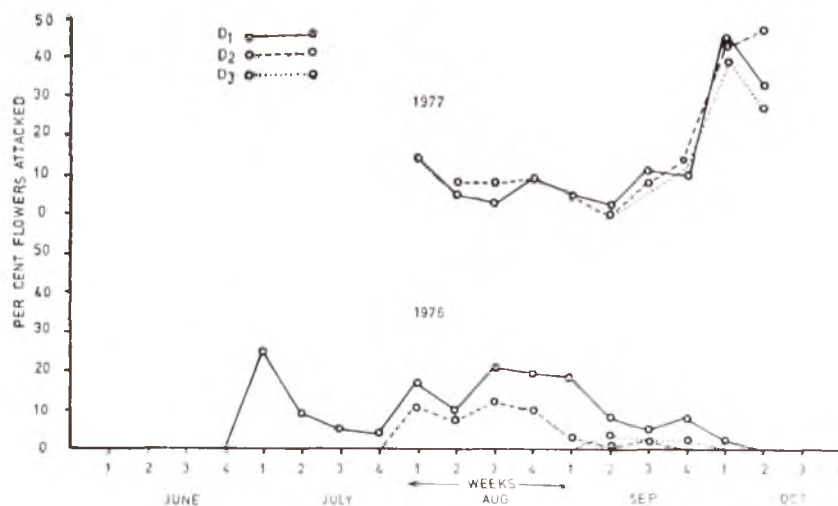
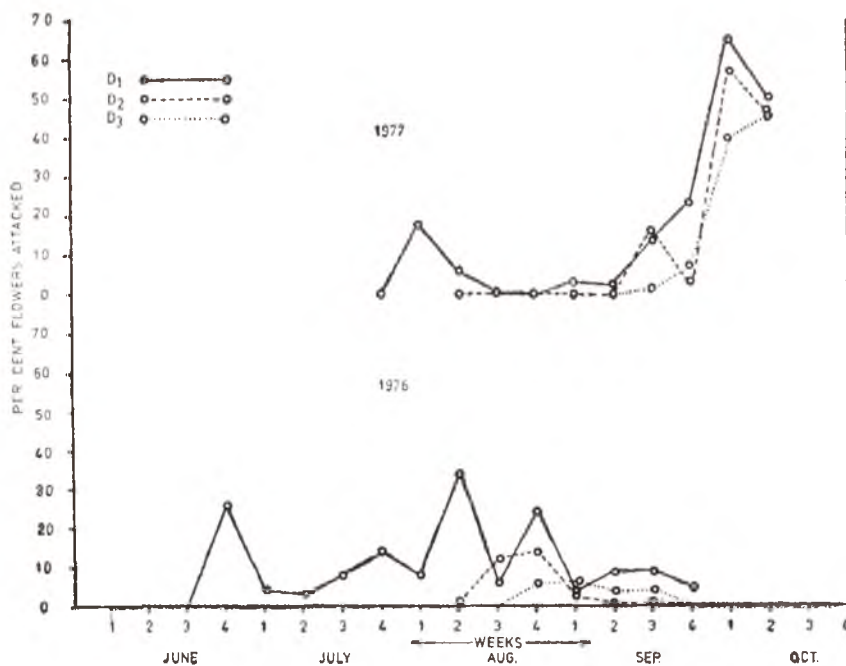
Sowing	F 414		J 205			
Dates	1975	1976	1977	1975	1976	1977
				<i>Period of pink bollworm attack*</i>		
D ₁	Aug. 24-Sept. 30	June 24 Sept. 30	Aug. 1 Oct. 15	July 16-Oct. 15	June 24-Oct. 7	Aug. 1-Oct. 15
D ₂	—	Aug. 1-Sept. 23	Aug. 8 Oct. 15	—	Aug. 8-Oct. 7	Sept. 16-Oct. 15
D ₈	—	Aug. 24-Sept. 23	Sept. 16-Oct. 15	—	Aug. 24-Sept. 23	Sept. 16-Oct. 15
				<i>Period of maximum pink bollworm attack*</i>		
D ₁	Aug. 1-7, 16-31	June 24-30, Aug. 8-15	Oct. 1-15	July 24 Aug. 15	June 24-30 Aug. 8-15, 24-31	Oct. 1-15
D ₂	—	Aug. 1-31	Oct. 1-15	—	Aug. 16-31	Oct. 1-15
D ₈	—	Sept. 8-15	Oct. 1-7	—	Sept. 1-7	Oct. 1-15
				<i>Per cent pink bollworm incidence (range)*</i>		
D ₁	0-52.00	0-25.40	0-44.50	0-92.30	0-33.60	0-65.10
D ₂	—	0-12.10	0-47.50	—	0-14.20	0-56.80
D ₈	—	0-4.80	0-40.10	—	0-6.40	0-45.90

*Based on 3 replications in 1975 and 4 replication in 1976 and 1977.

TABLE 5. Rosette formation due to pink bollworm attack in *hirsutum* varieties F 414 and J 205 in different years

Sowing date	F 414			J 205		
	1975	1976	1977	1975	1976	1977
	<i>Period of rosette-formation</i>					
D ₁	Aug. 24-Sept. 30	June 24-Sept. 23	Aug. 1-Oct. 15	July 16-Oct. 15	July 8-Oct. 7	Aug. 1-Oct. 15
D ₂	—	Aug. 1-Sept. 23	Aug. 8-Oct. 15	—	Aug. 8-Sept. 23	Sept. 16-Oct. 15
D ₃	—	Sept. 8-23	Sept. 24-Oct. 15	—	Aug. 24-Sept. 30	Sept. 24-Oct. 15
	<i>Period of maximum rosette-formation</i>					
D ₁	July 24-Aug. 7	June 24-30	Aug. 1-7 Oct. 1-7	July 24-Aug. 15	July 24-31; Aug. 8-15; 24-31	Oct. 8-15
D ₂	—	July 24-Aug. 23	Oct. 8-15	—	Aug. 16-31	Oct. 8-15
D ₃	—	Nil	Oct. 8-15	—	Nil	Oct. 8-15
	<i>Per cent rosette-formation during different periods*</i>					
D ₁	0-52.00	0-19.05	0-14.30	0-87.50	0-25.10	0-30.70
D ₂	—	0-12.10	0-20.70	—	0-11.70	0-29.30
D ₃	—	0-0.65	0-12.70	—	0-4.10	0-14.80
	<i>Per cent rosette-formation among the pink bollworm attacked flowers*</i>					
D ₁	0-100.00	0-96.40	14.60-100.00	0-100.00	0-90.50	0-74.80
D ₂	—	0-100.00	0-100.00	—	46.40-100.00	60.60-100.00

*Mean of 3 replications in 1975 and 4 replication in 1976 and 1977.

Fig. 4. Per cent flowers attacked by *P. gossypiella* in F 44.Fig. 5. Per cent flowers attacked by *P. gossypiella* in J 205.

The extent of flowers attacked by pink bollworm is reported to be 7.7 per cent in Madhya Pradesh (KAUSHIK *et al.*, 1960). This variation of pink bollworm attack may be due to different varieties and environment.

Period of resette-formation:

Resette-formation mainly occurred due to pink bollworm attack and all the rosetted flowers contained third or fourth instar

larvae. The period of rosette-formation, more or less, synchronised with the period of pink bollworm attack.

Period of maximum rosette-formation:

It was rather erratic but usually coincided with the periods of significantly more pink bollworm attack in flowers.

Extent of rosette-formation:

It varied with the variety, year and pink bollworm attack. Generally more rosettes were formed in *J* 205 than in *F* 414. All the attacked flowers did not transform into rosettes. Extent of rosette-formation on the basis of total flowers produced varied up to 52 per cent in *F* 414 against 87.5 per cent in *J* 205 during different periods. It was up to 100 per cent among pink bollworm attacked flowers in both the varieties.

Spotted bollworm incidence:

The incidence of *Earias* spp., among the flowers of both the varieties was less than that of the pink bollworm. It was up to 3.3 per cent in *F* 414 and up to 9.05 per cent in *J* 205.

According to KAUSHIK *et al.* (1969) *hirsutum* cotton suffered 3.19 per cent attack by spotted bollworm in Madhya Pradesh

which falls within the range observed in the present studies.

Flower-shedding:

The average of end-April sown crop during 1975 to 1977 (Fig. 3) revealed that maximum flower-shedding occurred due to physiological factors (other than the insects) in both the varieties. It was followed by spotted bollworm and pink bollworm respectively.

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INVESTIGATIONS ON *HELIOTHIS ARMIGERA* HUBNER IN MARATHWADA. II. THE RATES OF NATURAL INCREASE WHEN REARED ON SAFFLOWER *

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Heliothis armigera HUBNER is a polyphagous pest of many important crops. Studies for determining the rate of multiplication of its population were conducted at $26 \pm 1^\circ\text{C}$ temperature. Under conditions of abundant space and food supply, its population on safflower leaves increased with an infinitesimal rate of 0.1343 and finite rate of 1.1350 females per female per day. The population multiplied 358.53 times between two successive generations and the mean time for completing a generation was 43.60 days. On reaching a stable age-distribution, the population comprised approximately more than 99 per cent of immature stages. When mortality rate was 31 at the age interval of 45-50 days the expectation of further life was reduced to 1.99 days from 15.13 days in the beginning.

(Key words: *Heliothis armigera*, rate of increase, safflower)

INTRODUCTION

Heliothis armigera HUBNER is a serious and polyphagous pest feeding on many important crops of the Marathwada region. Life table is a concise summary of certain vital statistics of insect population. The innate capacity has been calculated for only one species of Noctuidae, the zebra caterpillar, *Ceramica picta* (TAMAKI *et al.*, 1972).

The purpose of this study was to determine the laboratory nutritional source that gave the greatest rate of increase of *H. armigera* when reared on safflower.

MATERIALS AND METHODS

The method used in this paper was similar to that given earlier (BILAPATE *et al.* 1977, 1978; BILAPATE & PAWAR 1978). In order to construct the life-tables, 100 eggs were placed in ten plastic

boxes in batches of ten each. The eggs were glued with the help of a soft wet camel hair brush on the white paper in one row to facilitate observations on hatching. After hatching, all the larvae of *H. armigera* were reared individually on safflower (*Carthamus tinctorius* LINN.) leaves. Fresh food was supplied daily. Observations on hatching, larval and pupal development, successful adult emergence, fecundity and age-specific mortality in eggs, larvae, pupae and adults were made daily. For determining the age-specific fecundity, the total number of adults emerged on a particular day were transferred to a separate cage for egg laying. The healthy safflower twigs, kept in the containers containing water were put into the cages as oviposition site. As the sex-ratio was 1:1 (based on 1500 adults) the number of eggs laid per female were divided by two to get the number of female births (m_x). The column headings proposed by BIRCH (1948), elaborated by HOWE (1953) and ATWAL & BAINS (1974) were used for the construction of life-fecundity tables under laboratory conditions.

X—pivotal age in days; l_x —survival of females at age X; m_x —age schedule for female births at age X; The values of X, l_x and m_x were calculated from the data on life-tables. The number of individuals survived at each age interval was recorded and also the mean number of female offspring produced at each age interval. From the data in life-table, the arbitrary values of r_m (r_c) was calculated. The

*Part of Ph.D. thesis submitted by senior author to Marathwada Agricultural University, Parbhani (MS) during March, 1979 and 19th contribution to the knowledge of *H. armigera* in the Marathwada region of Maharashtra State.

intrinsic rate of natural increase (r_m) was then calculated by using the life-table data, from the value of arbitrary r_m by taking two trial values arbitrarily selected on either side of it, differing in the second decimal place by interpolation method (BIRCH, 1948; WATSON, 1964). Tables were then constructed with the column X and $l_x m_x$ for each trial r_m . The two trial values of $\sum e^{r_m X} l_x m_x$ were then plotted on the horizontal axis against their respective arbitrary r_m 's on the vertical axis with the line drawn from the desired value of $\sum e^{r_m X} l_x m_x = 1096.6$. The point of the intersection gave the value of true r_m accurate to the four decimal places. The stable age-distribution was worked out with the knowledge of r_m and the age-specific mortality of the immature as well as mature stages. The life expectancy table was constructed according to the method of DEEVEY (1947) as summarised by SOUTHWOOD (1968) and ATWAL & BAINS (1974).

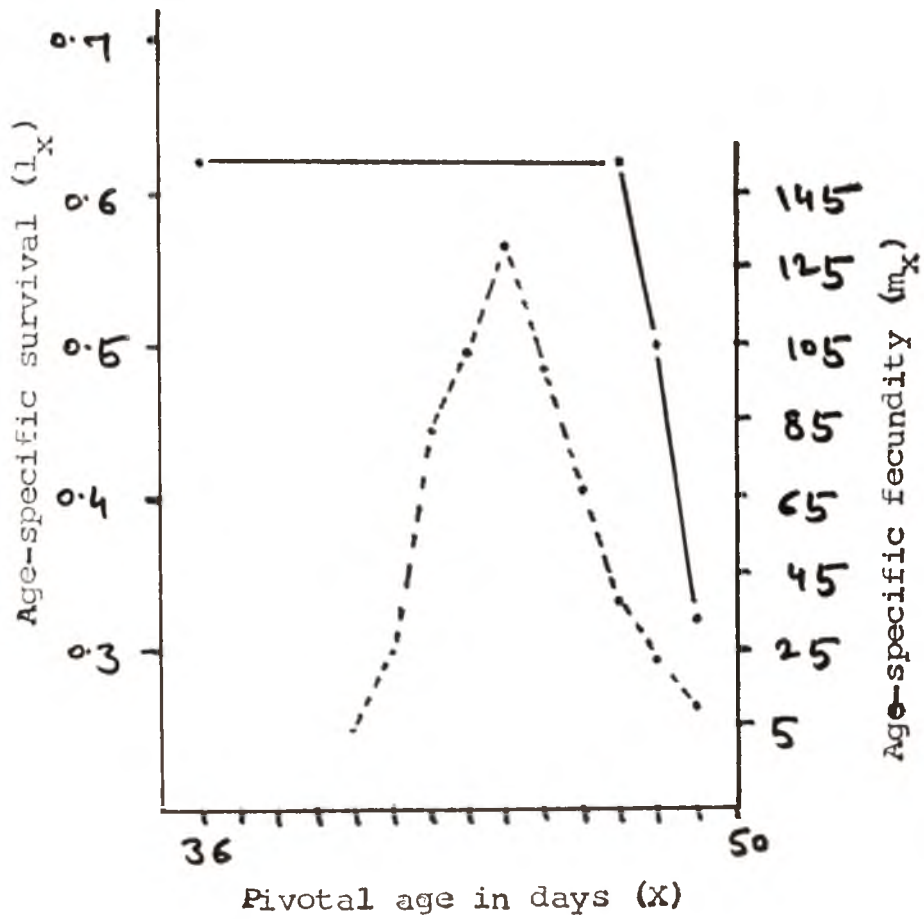
RESULTS

The results in Table 1 indicate that the maximum duration of egg, larva and pupa was 4, 16 and 15 days, respectively. The per cent hatch of egg was 90 per cent while

the successful larval pupation and pupae emerged into adults was 77.78 and 88.57 per cent, respectively. The results indicate that the first female mortality occurred on the 13th day after emergence and increased thereafter (Table 2). The net reproductive rate (R_0) representing the total female-births per generation was 358.53. The oviposition extended for ten days and reached a peak ($m_x = 130.30$ females per female per day) on 44th day of pivotal age and then declined (Fig. 1). It is obvious from Table 3 that the mean time for completing a generation (T) was 43.60 days. Thus, the innate capacity (r_m) and finite rate (λ) for increase in numbers were calculated as 0.1343 and 1.1350 females per female per day (Fig. 2). At this rate the population of *H. armigera* was capable to multiply 2.54 times per week under the set of conditions. It took 5.14 days to multiply the population in two-folds. The hypothetical F_2 females are 1,28,543.76. The

TABLE 1. Survival of different stages of *H. armigera* during development of safflower.

Egg kept	Number surviving		
	Egg stage 0-4 days	Larval stage 5-20 days	Pupal stage 21-35 days
10	10	8	6
10	10	8	6
10	8	7	7
10	10	7	6
10	9	7	6
10	9	7	6
10	9	7	7
10	9	7	6
10	8	6	6
10	8	6	6
100	90	70	62


 Fig. 1. Daily age-specific survival and fecundity of *H. armigera* on safflower.

..... Age specific fecundity (m_x)
 ————— Age specific survival (l_x)

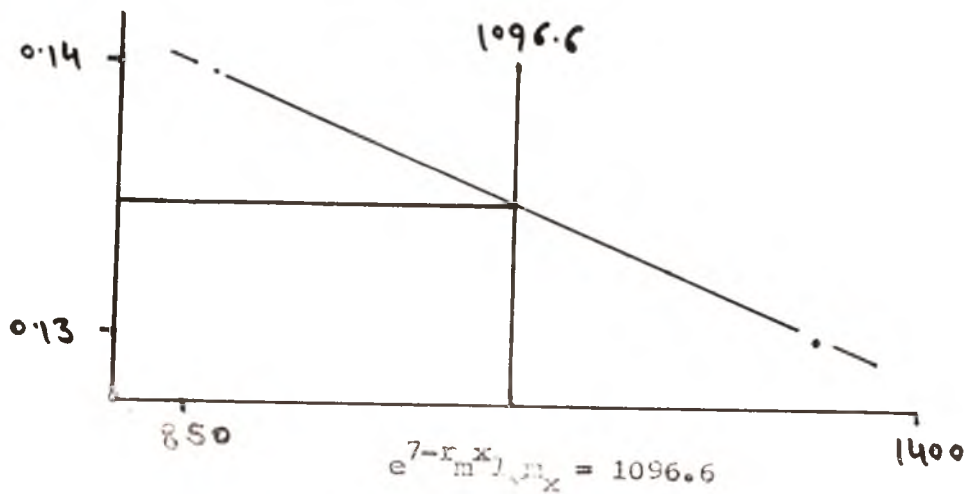

 Fig. 2. Determination of the intrinsic rate of increase (r_m) of *H. armigera* on safflower.

TABLE 2. Life table (for females) and age-specific fecundity for *H. armigera* on safflower.

Pivotal age in days	Survival of female at different age interval	Age schedule for female births		
X	l_x	m_x	$l_x m_x$	$l_x m_x X$
0-35	0.62	—	Immature stages	
36-39	0.62	—	Pre-oviposition period	
40	0.62	5.62	3.48	139.37
41	0.62	25.83	16.04	656.59
42	0.62	82.38	51.07	2145.17
43	0.62	103.25	64.07	2755.31
44	0.62	130.30	80.78	3554.58
45	0.62	100.80	62.49	2812.32
46	0.62	67.24	41.68	1917.68
47	0.62	39.27	24.34	1144.32
48	0.50	22.76	11.38	546.24
49	0.32	10.00	3.20	156.80
50	0.31	0.00	0.00	0.00
			$R_0 = 358.53$	$= 15828.38$
			$= \Sigma l_x m_x$	$\Sigma l_x m_x X$

TABLE 3. Mean length of generation, innate capacity for increase in numbers and finite rate of increase in numbers of *H. armigera* on safflower

Population growth statistics	
Mean length of a generation	
$T_c = \frac{\Sigma l_x m_x X}{R_0}$	44.15 days
Innate capacity for increase in numbers	
$r_0 = \frac{\log_e R_0}{T_c}$	0.1332 female/female/day
Arbitrary r_m 0.13 and 0.14	
Corrected $r_m \Sigma e^{-r_m X} l_x m_x = 1096.6$	0.1343 female/female/day
Corrected generation time	
$T = \frac{\log_e R_0}{r_m}$	43.60 days
Finite rate of increase in numbers	
$\lambda = \text{anti log}_e r_m$	1.1350 females/female/day
Weekly multiplication of population	2.54
Doubling time	5.14 days
Hypothetical F_2 females	1,28,543.76

TABLE 4: Calculated stable age-distribution of *H. armigera* on safflower ($r_m=0.1343$).

Pivotal age in days X	Lx	$e^{-r_m(X+1)}$	$Lx.e^{-r_m(X+1)}$	Percentage distribution	
1	2	3	4	5	
0	1.00	0.8743	0.8743	13.9586	
1	1.00	0.7644	0.7644	12.2040	
2	1.00	0.6683	0.6683	10.6628	53.04 eggs
	0.95	0.5843	0.5551	8.8625	
4	0.90	0.5109	0.4598	7.3408	
5	0.90	0.4467	0.4020	6.4181	
6	0.90	0.3905	0.3515	5.6119	
7	0.90	0.3415	0.3073	4.9062	
8	0.90	0.2985	0.2687	4.2899	
9	0.89	0.2610	0.2323	3.7088	
10	0.87	0.2282	0.1985	3.1692	
11	0.85	0.1993	0.1696	3.7078	
12	0.82	0.1744	0.1430	2.2381	32.64 larvae
13	0.80	0.1525	0.1220	1.9478	
14	0.78	0.1333	0.1040	1.6604	
15	0.76	0.1166	0.0886	1.4145	
16	0.74	0.1019	0.0754	1.2038	
17	0.72	0.0891	0.0641	1.0234	
18	0.70	0.0779	0.0545	0.8701	
19	0.70	0.0681	0.0477	0.7616	
20	0.70	0.0595	0.0417	0.6658	
21	0.70	0.0520	0.0364	0.5811	
22	0.70	0.0455	0.0318	0.5077	
23	0.70	0.0398	0.0278	0.4438	
24	0.70	0.0348	0.0243	0.3880	
25	0.70	0.0304	0.0213	0.3401	
26	0.70	0.0266	0.0186	0.2970	
27	0.69	0.0232	0.0160	0.2554	

1	2	3	4	5	
28	0.67	0.0203	0.0132	0.2107	
29	0.65	0.0177	0.0112	0.1788	
30	0.63	0.0155	0.0096	0.1532	
31	0.62	0.0136	0.0084	0.1341	3.87 pupae
32	0.62	0.0119	0.0074	0.1181	
33	0.62	0.0104	0.0064	0.1022	
34	0.62	0.0090	0.0056	0.0894	
35	0.62	0.0079	0.0049	0.0782	
36	0.62	0.0069	0.0043	0.0687	
37	0.62	0.0060	0.0037	0.0591	
38	0.62	0.0053	0.0033	0.0527	
39	0.62	0.0046	0.0029	0.0463	
40	0.62	0.0040	0.0023	0.0399	
41	0.62	0.0035	0.0022	0.0351	0.45 adult
42	0.62	0.0031	0.0019	0.0303	
43	0.62	0.0027	0.0016	0.0255	
44	0.62	0.0023	0.0014	0.0224	
45	0.62	0.0020	0.0013	0.0208	
46	0.62	0.0018	0.0011	0.0176	
47	0.56	0.0016	0.0008	0.0128	
48	0.41	0.0013	0.0005	0.0080	
49	0.31	0.0012	0.0003	0.0048	
50	0.31	0.0010	0.0003	0.0048	
6.2635				100.01	

stable age-distribution of *H. armigera* was worked out by observing the population schedule of birth-rate and death-rate (m_x and l_x) under the given set of conditions. It seems from the Table 4 that the adults contributed only 0.45 per cent to the population of stable age and the eggs, larvae

and pupae, 53.04, 42.64 and 3.88 per cent respectively. It is also seen that the population comprised approximately more than 99 per cent of the immature stages. When the mortality rate was 5 at age interval of 30-35 days, the life expectancy of further life reduced to 7.28 days from 15.13 days.

TABLE 5. Life-table for calculating life expectancy of *H. armigera* on safflower.

Pivotal age in days	Numbers sur- viving to the beginning of age interval out of 100	Numbers dying during age in- terval x	Mortality rate per hundred alive at beginning of age interval $\frac{d_x \times 100}{l_x}$	Alive between age x and x+1 $\frac{l_x + (l_{x+1})}{2}$	Numbers of the indivi- dual's life days beyond T_x	Expectation of further life $\frac{T_x}{l_x} \times 2$
X	l_x	d_x	$100q_x$	L_x	T_x	e_x
0-5	100	10	10.00	95.00	756.60	15.13
5-10	90	0.	0.00	89.80	661.60	14.70
10-15	90	1	1.11	89.50	571.80	12.71
15-20	89	11	12.36	83.50	482.30	10.84
20-25	78	8	10.26	74.00	398.80	10.23
25-30	70	0	0.00	70.00	324.80	9.28
30-35	70	5	7.14	67.50	254.80	7.28
35-40	65	3	4.62	63.50	187.30	5.76
40-45	62	0	0.00	62.00	123.80	3.99
45-50	62	32	50.00	46.50	61.80	1.99
50-55	31	0	0.00	15.30	15.30	0.99

DISCUSSION

BILAPATE *et al.* (1977, 1978) and BILAPATE & PAWAR (1978) studied the rate of multiplication of *H. armigera* on lucerne, lima bean and pea. The population increased with an infinitesimal rate of 0.1160, 0.1070 and 0.1346 females per female per day on lucerne, lima bean and pea, respectively. The net reproductive rates, were respectively 185.69, 206.47 and 407.39 females per female per generation. Thus, in the present investigations, safflower was superior to lima bean and lucerne. However, it was equally good when compared with pea in relation to reproductive rate and innate capacity for increase in numbers.

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MATING COMPETITIVENESS BETWEEN TWO STRAINS OF THE PINK BOLLWORM AND ITS ROLE IN DETERMINING DIAPAUSE IN THE PROGENY

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Mating competitiveness of pink bollworm. *Pectinophora gossypiella* males can be determined by tagging the release insects with a dye incorporated into the larval diet and checking the spermatophores in the females. In case of multiple matings the sequence can also be determined. In laboratory studies males of non-diapause Indian strain competed well with the males of the native diapause strain. Such matings did not result in a substantial decrease of diapause in the progeny.

(Key words: mating competitiveness, pink bollworm, *Pectinophora gossypiella* males, tagging, diapause)

INTRODUCTION

Large numbers of mass reared, sterilized pink bollworm, *Pectinophora gossypiella* moths have been routinely released in areas of California to suppress the native population of this serious pest of cotton (GRAHAM, 1978). In addition, the use of conditional lethal traits like the inability to enter diapause as a control measure has been suggested (KLASSEN et al., 1970). A common question associated with such release is, how competitive are these insects.

Lepidoptera in particular and in many insects the males transfer the sperm to the female in a membranous sac produced by the accessory glands of the former (WIGLESWORTH, 1965). Each successful mating is represented by the presence of a spermatophore in the female and if the males were tagged, the tag could be expressed in spermatophores transferred by such individuals. This study was undertaken to check how effective this method would be under labo-

ratory conditions and to do the preliminary evaluation of the use of a non-diapause strain in suppression of diapause.

MATERIALS AND METHODS

A non-diapause strain of the pink bollworm was obtained from India for laboratory testing of its capability to suppress diapause (RAINA & BELL, 1974).

Moths were obtained from larvae reared on artificial diet, according to the method described by BELL & JOACHIM (1976). Calco oil Red N-1700 dye at 0.01 % (w/v) was added to the larval diet and moths so obtained became tagged for most of their life (GRAHAM & MANGUM, 1971).

In the first experiment, single untagged females were alternatively mated to tagged and untagged males. The females were dissected after 3-4 matings and spermatophore sequence recorded. In the second experiment 20 untagged females of a diapause strain from Phoenix, Arizona (PS) were individually kept in cages (made from 450 ml icecream cups) with 2 males; 1 of the PS strain (also untagged) and 1 tagged male of the Indian strain (IS) for 7 days. Eggs were collected daily and the resulting larvae were tested for diapause incidence (under LD 12:12 and 18°C). After 7 days the females were dissected to check the number of the spermatophores and their sequence.

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TABLE 1. Representative samples from competitive matings between two strains of the pink bollworm and diapause response in the progeny*.

Sample type	Mating sequence and source of spermatophore			Per cent diapause among larvae reared from eggs laid on the following days of oviposition					
	1	2	3	1	2	3	4	5	6
1	PS	PS	IS	100	100	100	100	100	76
2	PS	IS	—	100	100	100	100	100	100
3	IS	PS	—	78	63	75	93	96	100
4	IS	PS	—	—	80	72	77	79	78

*Matings conducted in individual cages each containing 1 PS female and 1 each IS and PS males.

RESULTS AND DISCUSSION

Results from the first experiment indicated that the spermatophores from the tagged males carried the tag through the entire duration of the experiment.

Spermatophores were placed sequentially in the bursa copulatrix, and reflected the sequence of matings.

A close examination of the bursa indicated that the spermatophores were placed such that the most recent one had its chitinous neck lodged in the sperm duct. Muscular movements of the bursa wall cause 2 teeth like structures (signa) to press on the spermatophores and transfer the sperm to the spermatheca. With second mating, the first spermatophore was pushed back and the new spermatophore took its place with its neck in the sperm duct.

A representative sample of the results obtained from the second experiment is given in Table 1. In this test, males of both strains competed almost equally although PS males held a slight edge. There was no significant difference in egg laying and egg hatch between those that had mated with PS males and those with IS males. Females that had first mated with a PS male and later with an IS male produced 100% diapause progeny for the first 5-6 days. Incidence of diapause in the later progeny dropped to between 60-80%. The situation was reversed when the female first mated with an IS male followed by a PS male. It is presumed that the first mating took place on the first day but the time of subsequent mating(s) could not be ascertained.

Two things became evident from the present study: one, that the spermatophores are placed sequentially in the bursa and their source can be determined by tagging the males. Second, that the sperm from the first mating is not flushed out immediately after the second mating.

Several earlier workers (PAIR et al., 1977; ETMAN HOOPER, 1979) have reported sperm precedence of the last mating among some Lepidoptera. However, in case of the pink bollworm the sperm present in the spermatheca is used for few days before the female utilizes the sperm from the second mating. This is clearly reflected by the diapause incidence of the resulting progeny. It was also evident that the IS strain did not significantly suppress the diapause response in the progeny. Detailed studies on this aspect are reported elsewhere.

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A NEW SPECIES OF THE *MONTIUM* SUBGROUP OF GENUS *DROSOPHILA* (DIPTERA : DROSOPHILIDAE)

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(Received 22 March 1980)

A new species *Drosophila sampagensis*, a member of the *montium* subgroup of *melanogaster* species group collected from Sampage Ghats (Coorg District) is described. The taxonomic status and relationships are discussed.

(Key words: *Drosophila sampagensis*, new species, *montium* subgroup)

Judging from the reports on *Drosophila* taxonomy from other parts of the world, it appears that the number of species reported thus far from the Indian subcontinent is too small and is not commensurate with the luxuriant flora and diverse climatic conditions of the country providing many natural habitats for colonisation by *Drosophila* species. Still many parts await exploration to get a comprehensive knowledge on the *Drosophila* species inhabiting the subcontinent. In view of this the authors have chosen the unexplored area of the tropical rain forests of Coorg district situated on the summit of Western Ghats in the south-western part of Karnataka State between north latitude 11° 56' and 12° 50' and east longitude 75° 22' and 76° 11' to get an insight into the *Drosophila* species inhabiting this region. It is a picturesque high land clothed with primeval forests or grassy lands broken by a few cultivated valleys. This part of Western Ghats with its tropical flora and moderate to heavy rainfall offers an abode for the members of genus *Drosophila*. A maiden collection trip to Sampage Ghats, about 20 km to the west of Madakeri, has yielded several known species in addition to a new species, *Drosophila sampagensis* which is herein described.

Drosophila sampagensis, sp. nov.

Body length: Male 2.46 mm; Females 2.72 mm.

Head: ♂ and ♀ : Arista with 9 branches (5+4) including the terminal fork. Front light brown. Antenna dark brown. Basal segment of the antenna light yellow. Carina narrow. Palpi yellow and slender. Ocellar triangle small and light brown. Ocellar bristles long, proclinate. Inner verticals longer, outer verticals slightly shorter than inner ones. Orbital bristles in the ratio of 2:1:2. Eyes red.

Thorax: ♂ and ♀ : Brownish yellow, Acrostichal hairs in 8 rows, regularly placed. Ratio anterior: posterior dorsocentrals 0.5. Scutellum light brown. Anterior scutellars convergent, posterior scutellars crossed. Sternoidex 0.5. Pre-scutellars absent.

Legs: Pre-apical bristles on all tibiae. Apicals on first and second tibiae. Sex comb of male (Fig. 1) longitudinal, along the entire length of metatarsus and second tarsal segment. Metatarsal comb consisting of about 11 to 14 teeth, smaller above, longer below. Comb on second tarsal segment with about 5 to 9 uniform teeth.

Wings: ♂ and ♀ : Smoky and hyaline,



Fig. 1. Foreleg of male showing sex-combs.

C-index. 2.31; *4V-index.* 2.47; *5X-index.* 2.33; *M-index.* 0.9. Third costal section with heavy setation on basal 0.5. Wing lengths 2.76 mm (male); 2.96 mm (females).

Abdomen: ♂ and ♀ : Tergites of both sexes yellowish brown with dark apical bands which become darker in females with age.

Periphallic organs (Fig. 2) : Epandrium (Genital arch) broad dorsally and laterally; the round, heel with about 6 bristles. Primary and secondary surstyli present. Primary surstylus (primary clasper) broad, yellowish devoid of teeth but with a lateral row of about 6 irregularly arranged bristles, and a cluster of about 10 bristles on the lower border. Secondary surstylus (secondary clasper) fused with the cerci (anal plate), and carry 2 large curved black median teeth; the upper tooth is smaller than the lower. Cerci brownish, oval with about 16 bristles.

Phallic organs (Fig. 3): Aedeagus long apically pointed, recurved and bare. An-

terior gonopophyses (anterior parameres) triangular with hairy sensilla, not articulated to aedeagus. Posterior gonopophyses (posterior parameres) long, reaching the tip of the aedeagus. Caudal margin of novasternum with elongate median truncate processes, apically with a pair of submedian spines. Basal apodeme not projecting beyond the fragma.

Egg guide (Fig. 4): Brown with about 13 teeth and a subterminal hair.

Internal structures: Testes (Fig. 5) yellowish with 3 coils. Accessory glands large and transparent. Spermathecae (Fig. 6) vestigial. Paraovaria small, ventral receptacle long, tightly coiled. Malpighian tubules two pairs, free.

Egg filaments (Fig. 7): 2 long slender filaments.

Pupae: Black, anterior spiracle with about 8-9 branches.

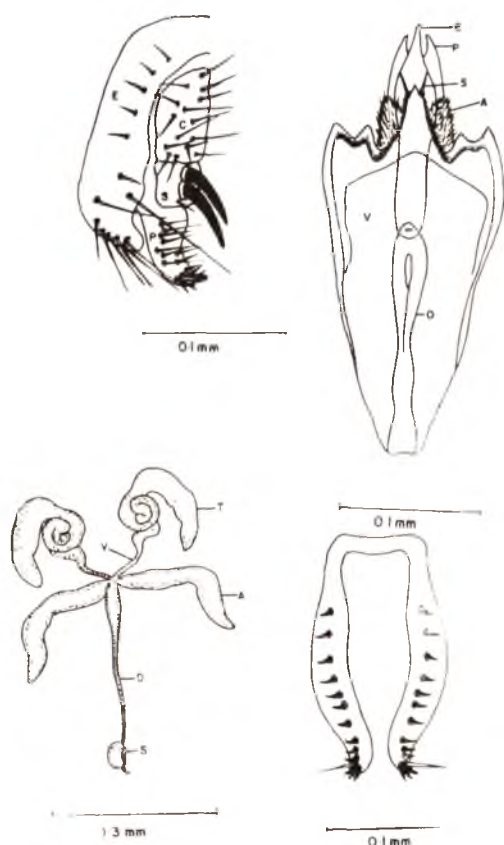


Fig. 2. (Upper left) Periphallallic organs; C—Cerci; E—Epandrium; P—Primary surstylus; S—Secondary surstylus. Fig. 3. (Upper right) Phallic organs. A—Anterior gonopophyses; E—Aedeagus; O—Basal apodeme of aedeagus; P—Posterior gonopophyses; S—Submedian spine of novasternum; V—Ventral fragma. Fig. 4. (Lower right) Egg guide. Fig. 5. (Lower left) Male reproductive organs. A—Accessory gland; D—Anterior ejaculatory duct; S—Ejaculatory bulb; T—Testes; V—Vas deferens.

Distribution: Coorg district (Western Ghats), Karnataka, India.

Taxonomic status: The nature of the banding pattern of abdominal tergites, sex comb pattern, structure of periphallallic and phallic organs, long coiled ventral receptacle and spiral testes qualify its inclusion in the *melanogster* species group of the subgenus

Sophophora. The yellowish abdominal tergites with distinct apical bands, presence of sex combs in male along the entire length of metatarsus and second tarsal segment, secondary sur-stylus with curved black median teeth permit its inclusion in the *montium* subgroup (Bock and Wheeler, 1972.)

Relationships and remarks: Okada (Personal Communication, February 1980) has pointed out that the new species resembles *D. barbata* (Bock and Wheeler, 1972) but differs from it in details. On comparison with other members of the *montium* subgroup it is found that the new species also resembles *D. mysorensis* (Reddy and Krishna Murthy, 1970) in the structure of periphallallic organs. However, the new species distinctly differs from both *D. barbata* and *D. mysorensis* in the intensity of pigmentation of apical bands in males, number and pattern of teeth in the sex combs and in the structure of anterior parameres. Further, the combination of characters such as the pattern and number of teeth in the sex combs, the structure of periphallallic and phallic organs are unique to this species and are not found together in any known species of the *montium* subgroup. Therefore, it deserves the status of a new species.

The new species can be cultured in the laboratory for two generations. The progenies obtained were very few and were used for the analysis of wing indices and other morphological characters.

The specific name *Drosophila sampagensis* is coined to denote the place, Sampage Ghats, where it was collected for the first time.

Holotype: ♂, INDIA, KARNATAKA, Coorg district (Western Ghats): Sampage Ghats, 12-i-1980. Coll. N. Muniyappa, G. Sreerama Reddy and H.S. Prakash. Deposited

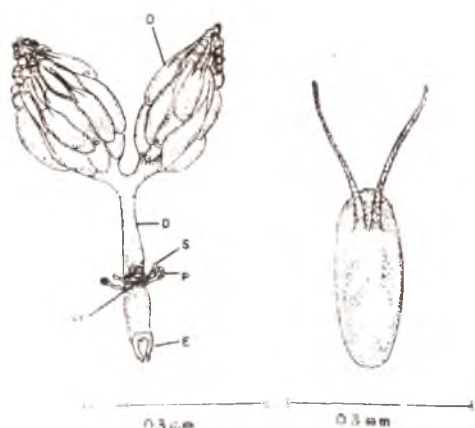


Fig. 6. (left) Female reproductive organs. D—Oviduct; E—Egg guide; O—Ovary; P—Paraovaria; S—Spermatheca; Vr—Ventral receptacle. Fig. 7. (right) Egg.

in the museum of the Department of Zoology, Manasa Gangotri, University of Mysore, Mysore. **Paratypes:** 10 ♂♂ and 10 ♀♀, data as above. Deposited in the Department of Biology, Tokyo Metropolitan University, Setagaya-ku, Tokyo, Japan and some will

be deposited in the Zoological Survey of India, Calcutta and Indian Agricultural Research Institute, New Delhi.

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APHIDS (HOMOPTERA: APHIDIDAE) OF NORTH WEST INDIA. VII: HITHERTO UNKNOWN MORPHS OF THREE SPECIES DESCRIBED FROM INDIA AND PAKISTAN

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Hitherto unknown apterous viviparous females of *Ceruraphis eastopi* Hille Ris Lambers infesting *Viburnum* sp. and *Eriosoma* (*Schizoneura*) *kashmiricum* Ghosh, Verma and Raychaudhuri infesting *Ulmus* sp. and alate viviparous females of *Melanaphis meghalayensis* meghalayensis Raychaudhuri and Banerjee infesting *Arundinaria* sp. are described for the first time from Garhwal, Himalaya, Uttar Pradesh, India.

(Key words: aphids, taxonomy, morphology, undescribed morphs, Uttar Pradesh, India)

1. *Ceruraphis eastopi* Hille Ris Lambers

(Figs. 1-4)

Material studied:

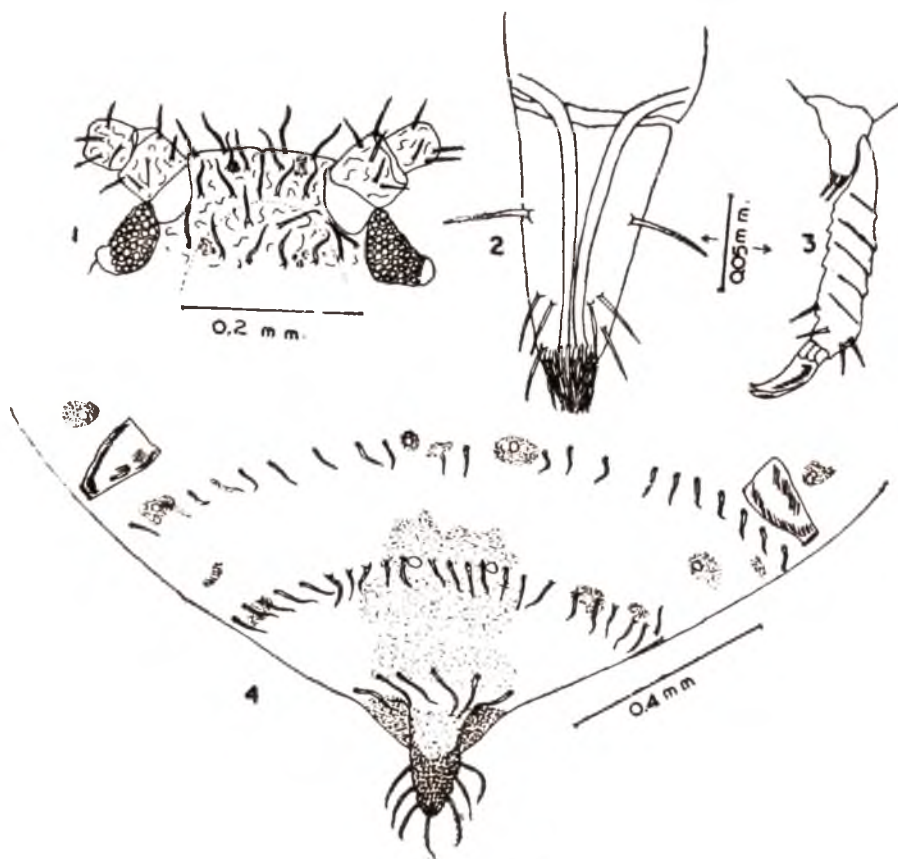
Many apterous viviparous females and nymphs, INDIA, UTTAR PRADESH, Ghangaria-12, vi. 1978 from pseudogalls of *Viburnum* sp. (coll. S. Chakrabarti).

Remarks: Hille Ris Lambers (1966) described *Ceruraphis eastopi* from West Pakistan only with alate viviparous females and pointed out that, when apterae are found they could be identified by the curious mixture of short and long hairs on the last two antennal segments of the embryo that they contain. The present collection of only apterae fulfil this diagnostic character and the identity of specimens was also confirmed by Dr. H.L.G. Stroyan, Harpenden, England.

Morphological characters:

Apterous viviparous female: Large sized aphids, about 3.42-3.84 mm long with 2.53-2.75 mm as maximum width.

Head blackish brown with ill developed lateral frontal tubercle; median frontal prominence moderately developed, slightly higher than antennal tubercles. Dorsal suture present, dorsum wrinkled having wax pores; hairs on the dorsum long stout with acute apices. Vertex with about 20 hairs including frontal tubercles, which are about 0.05-0.06 mm long and about 2.28-2.37 times as long as basal diameter of the antennal segment III; one pair of median dorsal tubercles present on the posterior margin of the head. Segments I and II blackish brown scabrous, particularly ventrally. I with 5 hairs and II with 3-4 hairs. Segment III blackish brown except the basal greater half, scatterdly imbricated, rest of the flagellum distinctly imbricated. Processus terminalis about 2.62-3.16 times as long as the base of the segment VI; flagellum without secondary rhinaria; longest hair on segment III about 1.25-1.57 times as long as the basal diameter of the segment. Ultimate rostral segment long blackish brown with 2 secondary hairs about 1.37-1.57 times as long as second segent of hind tarsus. Abdomen pale smooth with some scattered sclerotisation, anterior tergite with



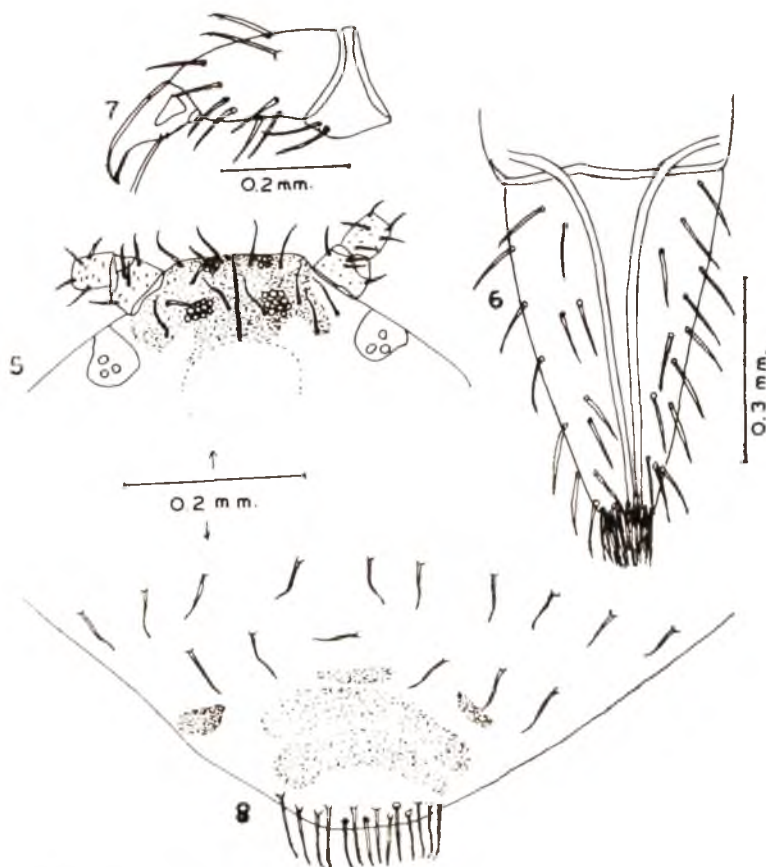
Figs. 1-4. *Ceruraphis eastopi* Hille Ris Lambers, Apterous viviparous female:
1. Head; 2. Ultimate rostral segment; 3. Second segment of hind tarsus;
4. Posterior portion of abdomen.

about 25-30 hairs, longest hair is about 2.71-2.75 times as long as basal diameter of segment III. 7th tergite with about 24 hairs and 8th with about 4-6 hairs, longest hair on 8th tergite is about 2.37-2.85 times as long as basal diameter of segment III; lateral tubercles present on 6th, 7th and 8th tergite, A pair of median tubercles present on 6th (often with 3 tubercles) and on 7th tergite. Siphunculi short subcylindrical, dark black brown in colour having poor imbrications and with a distinct flange, about 0.05-0.06 times as long as body and about 1.16-1.25 times as long as cauda; genital plate with 4 hairs on anterior margin and 22 hairs on posterior

margin arranged semicircularly; cauda black brown, elongate with a faint median constriction, with 6-8 hairs. Legs pale brown except apical 2/3rd portion of femora which is dark brown. First tarsal chaetotaxy 3,3,2.

Measurements of one specimen in mm :
Body length 3.42, width 2.53; antenna 1.16; antennal segments III: IV: V: VI 0.26:0.13: 0.16: (0.11+0.31); u.r.s. 0.16; h.t. 2 0.11; cauda 0.17.

Note : Aphids were collected from the



Figs 5-8. *Eriosoma (Schizoneura) kashmiricum* Ghosh *et al.* Apterous viviparous female: 5. Head; 6. Ultimate rostral segment; 7. Second segment of hind tarsus; 8. Posterior portion of abdomen.

pseudogalls of *Viburnum* sp., these galls are formed by folding of the leaf margin ventrally on both sides which touch the leaf blade ventrally reaching half way towards the midrib of the leaf.

2. *Eriosoma (Schizoneura) kashmiricum* Ghosh Verma and Raychaudhri (Figs. 5-8).

Material studied : One aptera, many alatae viviparous females and nymphs. INDIA: UTTAR PRADESH 10. vi. 1978, from galls of *Ulmus* sp. (coll. S Chakrabarti).

Remarks : Ghosh *et al.* (1976) described this species from alate viviparous females without assigning it to any subgenus.

Morphological characters : *Apterous viviparous female:* Body 2.78 mm long with 2.04 mm as maximum width. Head pale on wax plates regions otherwise dark brown; dorsum slightly rugose and with median suture; wax plates present both dorsally and ventrally, 1 pair present anteriorly and the other pair present posteriorly;

dorsum with 6 pairs of hairs with fine apices, longest hair on vertex about 55μ long and 1.66 times as long as basal diameter of the segment III. Antennae 5-segmented, brown, smooth except 0.30 portion of segment III and whole of segments IV and V which are spinulose, about 0.17 times the body; segments I and II slightly rugose with 4 and 5-6 hairs respectively; processus terminalis very short; flagellar hairs short with acuminate apices, longest one on segment III about 22μ long and 0.66 times the basal diameter of the segment. Rostrum reaching mid-coxae; ultimate rostral segment brown, about 2.34 times as long as second segment of hind tarsus and with 10 pairs of secondary hairs. Abdomen pale, dorsum weakly spinulose; dorsal hairs long with acuminate apices, longest one on anterior tergites about 37μ long and 1.10 times as long as basal diameter of segment III, 7th tergite with 10 and 8th tergite with 6 hairs and longest hair on these segments 40μ and 44μ long and 1.22 times and 1.33 times as long as the mentioned diameter, respectively; venter rather with prominent spinulosity. Siphunculi absent. Cauda broad and with 12-14 hairs. Legs brown, smooth; hairs on femora thin with fine apices; tarsi short. First tarsal chaetotaxy 2,2,2.

Measurements of the specimen in mm: Body length 2.78, width 2.04; antenna 0.47; antennal segments I: II: III: IV: V 0.06:0.06: 0.23: 0.06: (0.05 + 0.003); u.r.s. 0.14; h.t. 2,0 06.

Note: The leaf margin of the host plant (*Ulmus* sp.) folded ventrally and then twisted to form spiral gall from which the insects were collected.

3. *Melanaphis meghalayensis meghalayensis* Raychaudhuri and Banerjee.

Material studied: Many apterae, 4 alatae viviparous females and nymphs, INIDA, UTTAR PRADESH; Kedarnath. 3. vi. 1978 from *Arundinaria* sp. (coll. S.P. Maity).

Remarks: The apterous viviparous female of this species was described by Raychaudhuri and Banerjee (1974) from Meghalaya, Eastern Himalaya. This species is also recorded for the first time from North West Himalaya.

Morphological characters:

Alate viviparous female: Body about 1.51-1.62 mm long with 0.68-0.73 mm as maximum width. Head dark brown; dorsal cephalic hairs about 5.20-7.25 times as long as basal diameter of the segment III. Antennae about 0.65-0.70 times the body; processus terminalis about 2.57-3.0 times as long as the base of the segment VI; segment III with 18-24, IV with 4-8 secondary rhinaria distributed over the entire length of the segments; longest hairs on segment III about 3.60-5.0 times as long as the basal diameter of the segment; ultimate rostral segment about 0.75-0.81 times the second segment of hind tarsus. Abdomen pale, a continuous brown band present on tergites 3-5, dorsal abdominal hairs long with flagellate apices; a longest hair on anterior tergites about 5.0-6.50 times and on 7th and 8th tergites about 5.80-7.0 times and 6.20-7.50 times as long as basal diameter of segment III respectively. Siphunculi and cauda brown. Wing venation normal. Other characters as in apterous viviparous female.

Measurements of one specimen in mm: Body length 1.62, width 0.68; antenna 1.05; antennal segment III: IV: V: VI 0.29: 0.14: 0.14: (0.08 + 0.23); u.r.s. 0.06; h.t. 2,0.08; siphunculus 0.08; cauda 0.10.

Note : Black insects were collected from the growing shoots and leaves of *Arundinaria* sp. with waxy covering, associated with ants.

Acknowledgements:— The authors express their thanks to Dr. H.L.G. Stroyan, Herpenden, England for his comments on *Ceruraphis eastopi* Hille Ris Lambers and to the Head, Department of Zoology for laboratory facilities. Thanks are also due to the University Grants Commission for partially financing the work.

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A NEW SPECIES OF *DOLICHOVESPULA* AND SUBSPECIES OF *D. PACIFICA* (HYMENOPTERA : VESPIDAE) FROM CHINA

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Dolichovespula panda sp. nov. and *D. pacifica xanthicincta* ssp. nov. are described with their diagnostic characteristics and distributions. A new distribution record for *D. lama* (Buysson 1904) is given.

(Key words: *Dolichovespula*, Vespidae, new species, new subspecies)

This paper describes a new species *Dolichovespula panda* from female specimens. This new species is placed in the genus *Dolichovespula* Rohwer 1916 because it has a long oculo-malar space (Fig. 1), longer than the terminal diameter of the scape, and by the presence of well-developed pronotal carina.

The names of institutions in which material is housed are herein abbreviated as follows: BMNH—British Museum Natural History, London. USNM—National Museum of Natural History, Washington. ZSI—Zoological Survey of India, Calcutta.

Fig. 1 shows the head measurements that have been made.

1. *Dolichovespula panda* sp. nov. Queen and worker.

Head yellow with markings as follows: vertex and dorsal part of ocular sinus black; brown area between vertex and gena; sometimes a black stripe from antennal bases to vertex; scape with dorsal brown stripe; pedicel and flagellum dorsally black.

Thorax and propodeum black with markings as follows: pronotum yellow-marked dorsally on carina and ventrally in front of carina; tegula dark brown with

anterior and posterior yellow spot; meso-scutellum with posterior margin brown; fore-leg with coxa yellow or light brown ventrally, femur outermost dark brown and innermost light brown becoming yellow apically, tibia yellow with outer brown spot; middle and hind legs black to dark brown; tarsi on all legs dorsally dark and ventrally light brown.

Gaster: first, second, third tergites black sometimes third tergite with a lateral yellow spot; fourth tergite varying from black with a narrow yellowish brown apical band to largely yellowish brown with black restricted to a narrow basal band and marginal baso-apical stripe and sometimes a lateral black spot which may be attached to the basal black band; fifth tergite either entirely yellowish brown or with a black lateral baso-apical stripe; sixth tergite either entirely yellowish brown or with a central basoapical black stripe; first, second, third, fourth sternites black or dark brown and then second, third, fourth sternites may have a lateral black spot; fifth sternite basal half black, apically either yellowish-brown with a marginal baso-apical black stripe or dark brown with a lateral black spot; sixth sternite yellowish brown.

Hairs long and black but pale on: inner ocular sinus, ventral clypeus, mandible, posterior gena, ventral pronotum, around

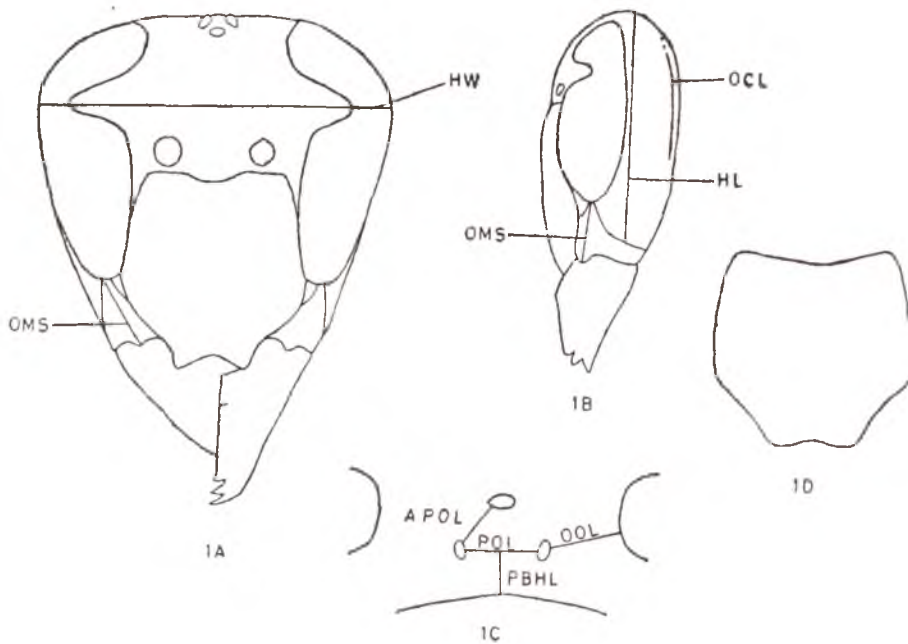


Fig. 1. Head of queen *Dolichovespula panda* sp. nov. (A—Frontal view, B—Lateral view, C—Vertex) and clypeus of worker *D. pacifica xanthicincta* ssp. nov. (D). OCL—occipital carina length, HL—head length, POL—postocellar line, OOL—ocellar-ocular line, APOL—anterio-postocellar line, PBHL—postocellar back of head line, HW—head width, OMS—oculomalar space.

base of wings, metanotum, mesepisternum, mesepimeron, dorsal and ventral metapleura, propodeum, fourth to sixth gastral segments. Long pale hairs on coxa, trochanter, femur, tibia.

Oculo-malar space very long (HW/OMS, ♀ 5.12, ♂ 5.32); clypeal large punctures present on disc with inter-puncture distance greater than puncture diameter except apically where the punctures are closer together; clypeal angles strongly projecting and bluntly triangular; POL/OOL, ♀ 0.5, ♂ 0.4, POL/APOL, ♀ 1.4, ♂ 1.6; POL/PBHL, ♀ 0.7, ♂ 0.7; margin behind inner mandibular tooth at first concave and then straight; occipital carina not clear in ♂ but it does not reach the base of the mandible in ♀ OCL/HL, 0.51; pronotal carina well-developed; pronotum punctate; mesoscutal coarse punctures with inter-puncture distance

about twice puncture diameter; mesoscutal micropunctures with distinct interspaces; propodeum punctate becoming weakly striate ventro-laterally; ventral metapleura punctate; dorsal margin of sixth gastral sternite concave.

Body length: ♀ 17.5 mm, ♂ 12.3-14.7 mm.
Forewing length: ♀ 14.6 mm, ♂ 11.1-12.4 mm.

Material examined: **Holotype** ♀, CHINA, Szechuen, 14.4 km south-west Tarsienlu, 2600-4000 m, June 1923 (D. C. Graham) (USNM). **Syntypes**, CHINA: ♂, no data (K. Richardson) (BMNH); 2♂, Szechuen, Suifu, 300-450m June 1928 (D.C. Graham) (USNM, BMNH); ♂, Szechuen, between Fu Yao Linn Pass and Da Shiang Lin Pass, 2000 m, Aug. 1923 (D. C. Graham) (USNM).

D. panda may be separated from other species of *Dolichovespula* by the following combination of characters: oculo-malar space very long (Fig. 1); anterior angles of clypeus greatly projecting but rounded (Fig. 1A); punctures on clypeal disc large and 2-3 puncture diameters apart; ventral pronotum lacks striations.

D. panda comes very close to *D. lama* (Buysson 1904) but may be separated by colour characteristics. *D. lama* has a dorsal yellow stripe on the pronotum, lateral yellow spot on the mesoscutellum and metanotum and yellow bands on the first three gastral tergites and sternites. These areas are all black on *D. panda*. Unlike *D. panda*, *D. lama* lacks punctures on the disc of the clypeus but more specimens need to be examined to confirm this character. The holotype queen *D. lama* (Skikim. C. T. Bingham, BMNH) was examined to make these comparisons.

Distribution: *D. panda* is found in Szechuen, China while the closely related *D. lama* is found further west in Sikkim (Buysson 1904) and in the Garhwal district, Uttar Pradesh, India (♀, 3200 m. June 1965, ZSI).

2. *Dolichovespula pacifica xanthicincta* ssp. nov. Worker.

Head black with markings as follows: frons with yellow shield extending to antennal bases; ocular sinus with narrow ventral yellow stripe extending to clypeal border; clypeus with lateral broad yellow stripe; mandible yellow except for ventral margin; gena with anterior dorsal and ventral yellow spot, ventral spot sometimes larger; antennal scape dark brown dorsally and yellow ventrally, pedicel and flagellum light brown ventrally.

Thorax and propodeum black with markings as follows: pronotum with narrow

parallel-sided yellow stripe; tegula light brown sometimes with an anterior and posterior yellow spot; mesoscutellum with two anterior rectangular yellow spots, sometimes quite small; metanotum with two antero-lateral small yellow spots; coxa and trochanter dark brown sometimes coxa with a ventral yellow spot; femur basally brown becoming light brown and yellowish apically; tibia and tarsus yellowish light brown with an outer brown spot on foretibia.

Gaster black with narrow apical yellow bands on all tergites and sternites except first sternite.

Body hair long and black except pale on: ventral pronotum, around base of wings, metanotum, mesepisternum, mesepimeron, dorsal and ventral metapleura, propodeum, vertical face of first gastral tergite, all gastral sternites. Long pale hairs on coxa trochanter, femur, tibia.

Oculo-malar space very long (HW/OMS, 5.5); clypeal coarse punctures with inter-puncture distance at least three puncture diameters apart except laterally and apically where punctures are closer together; clypeal angles semicircular; POL/OOL, 0.6; POL/APOL, 1.6; POL/PBHL, 1.5; margin behind inner mandibular tooth at first concave and then straight; OCL/HL, 0.5; pronotal carina well-developed; pronotum punctate; mesoscutal punctures with interpuncture space about twice the puncture diameter; mesoscutal micropunctures with distinct interspace; propodeum punctate becoming weakly striate ventro-laterally; ventral metapleura with indistinct punctures; dorsal margin of sixth gastral sternite convex.

Body length: 11.7-13.7 mm.

Forewing length: 10.4-12.2 m.

Material examined: Holotype ♂, CHINA-TIBET BORDER, near Tang Gu, 4300 m, Aug. 1930 (D. C. Graham (USNM)). Syntypes,

64♂, data as for holotype (63 ♂ USNM, 1 ♂ BMNH); TIBET, ♂ Mi-Chi, Yu Long Si Gge, 4000-4600 m, Aug. 1930 (D. Graham) (BMNH); NORTH BURMA, ♂ Adung Valley, 3700 m, Aug. 1931 (Lord Cranbrook) (BMNH).

The new subspecies *D. pacifica xanthicincta* is described from worker specimens. Guiglia (1972) and Yamane (1975) give keys which will separate *D. pacifica* (Birula 1930) from other Palaearctic species of *Dolichovespula* except for *D. lama* and *D. panda*. The clypeal angles are less projecting on *D. pacifica* (Fig. 1D) and the POL is larger than the PBHL but smaller on *D. lama* and *D. panda* (Fig. 1C). *D. pacifica xanthicincta* differs from the typical subspecies in that white markings are replaced by yellow.

Distribution: Typical *D. pacifica*—Eastern Siberia; Kamchatka; Sakhalin; Chishima; Japan (Hokkaido, Honshu, Shikoku) (Yamane 1975). *D. pacifica xanthicincta*—North

Burma; Tibet; Tibet-China border. Thus present distribution records indicate the subspecies do not overlap and the typical subspecies is the northern form.

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Throughout this article the symbol '♂' stands for worker.

BRIEF COMMUNICATION

CHANGES IN ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE ACTIVITY IN THE NERVOUS SYSTEM DURING MOULTING IN THE SILKWORM *BOMBYX MORI* L.

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(Received 16 May 1980)

Acetylcholinesterase (AChE, EC. 3.1.1.7) and butyrylcholinesterase (BuChE, EC. 3.1.1.8) activity changes in the brain and ventral nerve cord during moulting in the silkworm *Bombyx mori* L. were studied. Investigations made during an early stage moult, second, and a late stage moult (third) indicate that enzyme titres in the brain as well as in the nerve cord exhibit changes during moulting. The trend of changes is similar in both the regions although the magnitude of changes is different.

(Key words: acetylcholinesterase, butyrylcholinesterase, nervous system, moulting, *Bombyx mori*)

The powerful neurotoxic action of nicotine on insects has long suggested that important phases of synaptic transmission in insect central nervous system are cholinergic in nature (ROEDER, 1958). The occurrence of acetylcholinesterase (AChE) and its correlation to electrical activity has been reported in *Periplaneta americana* (MIKALONIS & BROWN 1941). Emphasis has been made that acetylcholine is the only choline ester present in *Periplaneta americana* (CHANG & KEARNS 1955) that was later confirmed (COLHOUN & SPENCER 1959). Occurrence of acetylcholine-like substances along with cholineacetylase and cholinesterase has been shown in the eggs of *Bombyx mori* (CHINO, 1957). Changes in acetylcholine levels and specific AChE activity in the central nervous system during metamorphosis have also been reported (PRESCOTT *et al.*, 1977; HABIBULLAH & NEWBURGH, 1973). In the present investigation a study of the changes in the activity of cholinergic enzymes AChE and BuChE in the nervous system during moult cycles in *Bombyx mori* L. has been made. Since moulting also like metamorphosis is under hormonal

control triggered by the neurosecretions of the brain, such a study was appealing.

Cellular and disease free egg layings were used. The silkworms were reared under photoperiod of 16L:8D (5.4 lux) with a relative humidity of 80–85% and a temperature of $26 \pm 2^\circ\text{C}$ during the early instars and a relative humidity of 70–80% and a temperature of $23 \pm 1^\circ\text{C}$ during the late instars. The stages chosen for study were before, during, and after second and third moults. Enzyme assays were performed by the method of ELLMAN *et al.* (1961). Substrates used were acetylcholine chloride and butyrylthiocholine chloride and the activity expressed as specific activity (nm substrate hydrolysed/mg pr/min). The results were statistically analysed. Protein was determined by the method of LOWRY *et al.* (1951).

During second moult the AChE titre increased by 90% in the brain and by 118.8% in the nerve cord while during the third moult enzyme increased by 11.57% and 83.8% respectively when compared to the

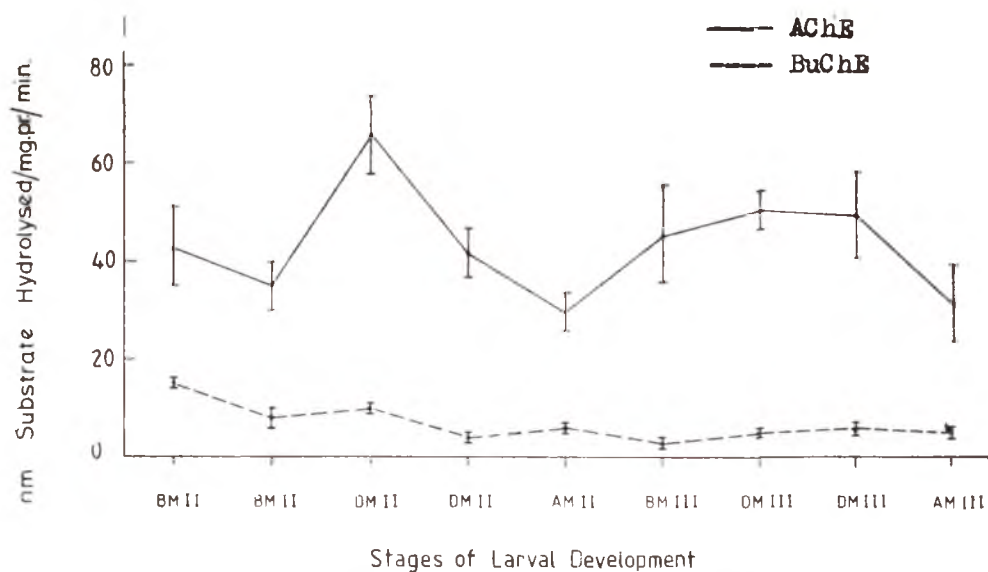


Fig. 1 Changes in activity of acetylcholinesterase and butyrylcholinesterase in the brain during second and third moult cycles.

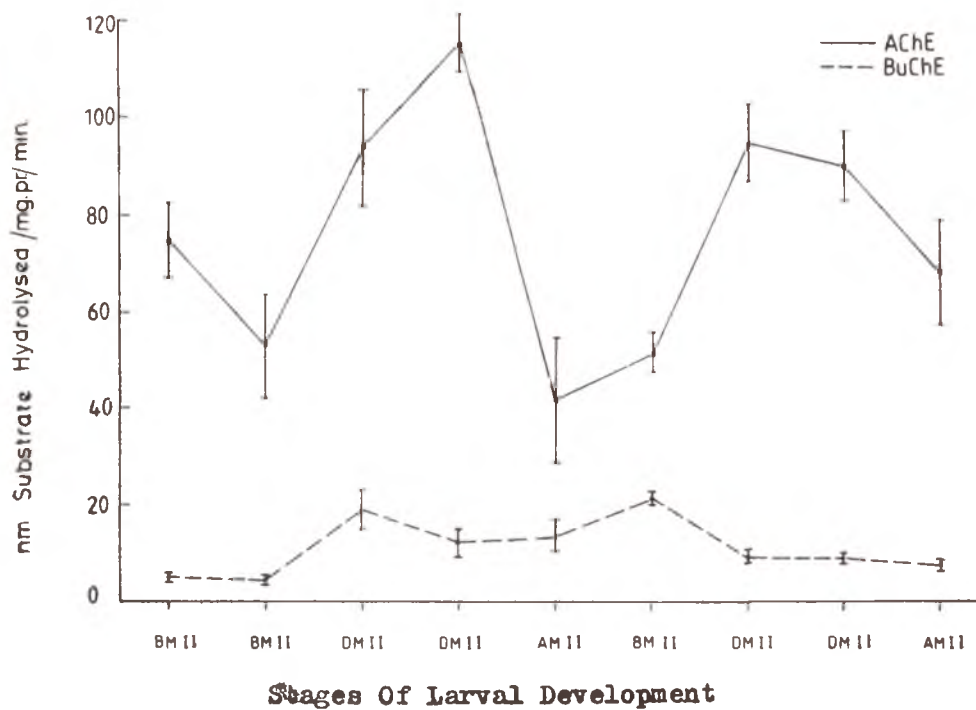


Fig. 2 Changes in activity of acetylcholinesterase and butyrylcholinesterase in the nerve cord during second and third moult cycles.

Abbreviations: BM—Before moult. DM—During moult. AM—After moult.

values before moult. Specific activities of AChE and BuChE, stood at a lower level in the brain than in the nerve cord. In the brain the specific activity of AChE was 53.3% of that in the nerve cord while the specific activity of BuChE was 60.8% (Figs. 1,2). During second moult, specific activity of AChE in the brain was found to be 57.1% and during third moult it was found to be 53.4% of that of nerve cord. The activity of BuChE was far less compared to the activity of AChE forming 16.1% of that in the brain and 15.7% of that in the nerve cord emphasising the greater involvement of AChE in neuronal transmission in *Bombyx mori* L.

Changes in the enzyme levels in the brain as well as nerve cord during moulting indicates that unlike in *Platysamia cecropia* and *Hyalophora cecropia* (VAN DER KLOOT, 1955) changes occurring during moulting differed from those occurring during diapause although apparently under both conditions the animals were inactive and nonfeeding. The brains of *Platysamia cecropia* exhibited an electrical silence, absence of AChE and a consequent accumulation of ACh, while the ventral nerve cord exhibited activity throughout the period of diapause. The increased AChE activity during moult suggested that changes in activity during moulting did not occur in accordance with the locomotor activity in this animal, as it actually had minimal locomotor activity in this stage. Increase in ChE activity during diapause of oak silkworm has also been reported (TYSHTCHENKO & MANDELSTAM, 1965). It is possible that the increased AChE activity during diapause in the oak silkworm and during moult in the present investigation are more related to morphological and functional reconstructions of the nervous system.

Moulting and metamorphosis are under the control of the hormones ecdysone and

juvenile hormone (JH). As suggested by VAN DER KLOOT the reappearance of AChE is elicited by high substrate concentrations (cholinergic substance acetylcholine accumulated during moulting) which consequently makes the brain electrically active enabling the neurosecretory cells to release hormones. This event also goes as an evidence for the fact that hormone release is under nervous control (FINLAYSON *et al.*, 1976).

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BRIEF COMMUNICATION

RELATIVE TOXICITY OF DIFFERENT CHEMICALS FOR THE CONTROL OF RED SPIDERMITE, *TETRANYCHUS CINNABARINUS* (BOISDUAL)(*T. TELARIUS* L.) ACARINA : TETRANYCHIDAE, ON CASSAVA

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(Received 22 March 1980)

Studies using eight insecticides revealed that monocrotophos, dicrotophos, dimethoate and methyl demeton were effective in suppressing red spider mite *Tetranychus cinnabarinus* population on cassava.

(Key words: chemical control, red spider mite, *Tetranychus cinnabarinus*)

INTRODUCTION

The most common and wide-spread pest of cassava in Kerala is the red spider mite, *Tetranychus cinnabarinus* (*T. telarius*). Although the pest is found throughout the year, heavy infestation occurs during hot and dry seasons (RAO & PILLAI, 1973). The pest is mostly confined to the under surface of the leaves. In case of heavy infestation they move towards the upper surface also and sometimes the leaf lobes are seen webbed together. In view of the seriousness of the problem a field experiment was conducted using different chemicals and the results are reported here.

The experiment was conducted at the farm of the Central Tuber Crops Research Institute, Trivandrum during 1976 using the variety H-97. Experiment was in RBD consisting of eight treatments and control in three replications (vide Table I). There were 24 plants per replication. Pre-treatment population counts were taken from 6 plants of middle two rows per replication, observing 3 leaves one each from lower, middle and top portions. Post-treatment observations 2, 7, 15 and 30 days after spraying were taken and the mortality worked

out. The mortality obtained in different treatments at different intervals was recorded and the corrected mortality in percentage was computed by the formula of HENDERSON & TILTON (1955).

Table I shows the corrected per cent mortality and their transformed values in different treatments at different time intervals. The mortality percentages in different treatments at 2, 7, 15 and 30 days intervals were found to be 35.33 to 91.90, 40.00 to 93.20, 46.80 to 96.20 and 21.50 to 88.50 respectively. The toxicity of different chemicals after 30 days was found in the descending order of monocrotophos > dicrotophos > dimethoate > chinomethionate > methyl demeton > phosphamidon > leptophos and > carbaryl. In all time intervals monocrotophos was most effective, but statistically dicrotophos, dimethoate, methyl demeton and chinomethionate were found at par with monocrotophos. The treatments phosphamidon, leptophos and carbaryl were found significantly inferior to others. Phosphamidon was found effective only upto 2 days and thereafter its toxicity declined and became almost at par with leptophos and carbaryl.

TABLE 1. Showing the per cent mortality in different treatments at different time intervals.

Insecticides and concentrations(%)	Per cent mortality at the end of: (after spraying)					Cumulative effect
	2 days	7 days	15 days	30 days		
Phosphamidon	0.03	88.43 (70.41)	65.30 (54.77)	73.10 (59.77)	49.10 (44.18)	68.96 (57.36)
Dimethoate	0.03	86.86 (68.86)	85.26 (67.95)	92.50 (74.23)	86.13 (68.30)	87.68 (69.33)
Dicrotophos	0.05	91.33 (73.30)	75.96 (60.75)	92.80 (74.66)	88.50 (70.41)	87.14 (69.78)
Monocrotophos	0.05	91.90 (73.58)	93.20 (75.33)	96.20 (78.77)	88.20 (71.15)	92.53 (74.71)
Methyl demeton	0.05	91.10 (72.68)	87.76 (70.48)	90.60 (72.69)	84.30 (66.88)	88.43 (70.68)
Leptophos	0.05	68.80 (56.58)	42.80 (40.76)	52.90 (46.69)	36.70 (36.70)	50.12 (45.18)
Chinomethionate	0.1	86.33 (68.55)	87.20 (69.83)	83.70 (67.27)	85.70 (68.05)	85.71 (68.43)
Carbaryl	0.1	35.33 (36.26)	40.00 (39.21)	46.80 (43.18)	21.50 (25.73)	35.90 (36.09)
C D at 5%		9.38	14.01	11.65	14.22	5.61

Figures in parenthesis are transformed values

Combined analysis for the cumulative effect also revealed that monocrotophos, dicrotophos, dimethoate, methyl demeton and chinomethionate were significantly superior to rest of the chemicals (Table 1). PILLAI (1968) reported the efficacy of dime-methoate for the control of red mite. SARADAMMA *et al.* (1972) found carbofuran, monocrotophos, dimethoate and formothion as effective against mites. In the present investigation, monocrotophos, dicrotophos, dimethoate and methyl demeton were found highly effective and phosphamidon, leptophos and carbaryl were ineffective to suppress the red mite population on cassava. This observation agrees with the earlier report of the efficacy of monocrotophos and dime-thoate against red spider mite.

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VARIETAL SUSCEPTIBILITY OF WHEAT VARIETIES TO BROWN WHEAT MITE *PETROBIA LATENS* (MULLER) (ACARINA : TETRANYCHIDAE)

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An experiment was conducted to test the comparative susceptibility of different wheat varieties to brown wheat mite. The different varieties showed significant differences in mite population. The variety WL 410 harboured minimum and variety WG 1809 the maximum number of mites.

(Key words: wheat, brown wheat mite)

Brown wheat mite *Petrobia latens* (MULLER) is a cosmopolitan pest of wheat. In India it was first reported from Madhya Pradesh by BINDRA & KITTUR (1961) and from other parts of the country (MENON & GHAI, 1968; PHADKE *et al.*, 1972). The mite attacks the lower leaves first, and slowly travels upwards. The damage appears in the form of minute white specks on the leaf surface. Severely damaged leaves start drying from the tip. Highly infested crop gives a palish appearance. The attack of this mite has been found to be more serious on unirrigated than that on the irrigated crop. A trial was, therefore, conducted to screen wheat varieties under unirrigated conditions. The screening was done when the mite infestation was heavy. The results are reported here. In all 46 varieties of wheat were tested. All the varieties were sown in a randomized block design with four replications. The plot size for each variety was 6 rows, 23 cm apart and 6 m long. The population of the mites was recorded at random from five clumps per replication, the two side rows of the plots not being considered. The population was recorded by "Jarring Method" (DEOL & SANDHU, 1974), which consist of six quick tappings

to plant by hand to dislodge the mites on a white paper (30 cm × 14 cm). The mites thus collected were transferred to glass vials containing 70 per cent alcohol. All the active stages were counted under a stereoscopic microscope in the laboratory. The data were statistically analysed. The results are presented in Table 1. Variety WL 410 was found to be highly resistant as it harboured the minimum (211.75) number of mites. Variety WG 1809 was the most susceptible having the maximum population (1489.75) of mites. The data showed significant differences in mite population on various varieties. Varieties WG 1550, WH 242, WL 711, UP 2029, DL-39-5, WL 2357, WG 1552, UP 2051, WL 2197, IWP 72, C 518, UP 2052 showed resistance to the mite; varieties WL 2350, HP 1345, WL 2206, HS 81, Raj 1634, WL 2203, WG 1810, Raj 1655, Raj 1491, K 7792, BH 4281, UP 1002, WH 271, HD 2280, WL 2201, were moderate in reactions and varieties UP 196, DL-79-1, WH 275, Raj 1592, BH 4371, WH 277, WH 274, WH 272, WG 1808, WH 273, Raj 1627, BH 4337, WH 276, K 7793, C 306, Raj 1626, WL 1541, WG 1809 were susceptible to the pest as these harboured high population of the mite.

TABLE I. Incidence of *P. latnes* on different varieties of wheat.

Sl. No.	Variety	Mean number of mites present/5 clumps
1.	WL 410	211.75 (14.52)
2.	WL 711	350.25 (18.67)
3.	WL 1541	1393.75 (37.07)
4.	WL 2197	447.25 (20.93)
5.	WL 2201	888.75 (29.76)
6.	WL 2203	749.75 (27.32)
7.	WL 2206	705.75 (26.41)
8.	WL 2350	612.25 (24.71)
9.	WL 2357	409.75 (20.16)
10.	WG 1550	297.0 (17.04)
11.	WG 1552	412.25 (20.25)
12.	WG 1808	1097.5 (33.09)
13.	WG 1809	1489.75 (38.57)
14.	WG 1810	791.25 (28.04)
15.	WH 242	338.75 (18.30)
16.	WH 271	870.75 (29.44)
17.	WH 272	1066.75 (32.54)
18.	WH 273	1185.0 (34.39)
19.	WH 274	1022.0 (31.94)
20.	WH 275	984.25 (31.34)
21.	WH 276	1293.0 (35.90)
22.	WH 277	1004.25 (31.67)
23.	UP 196	940.5 (30.60)
24.	UP 1002	870.0 (29.41)
25.	UP 2029	355.5 (18.93)
26.	UP 2051	444.50 (21.04)
27.	UP 2052	517.25 (22.59)
28.	Raj 1491	814.5 (28.48)
29.	Raj 1592	988.0 (34.74)
30.	Raj 1626	1388.75 (37.23)
31.	Raj 1627	1194.75 (34.49)
32.	Raj 1634	748.5 (27.20)
33.	Raj 1655	792.5 (28.09)
34.	BH 4281	832.15 (28.58)
35.	BH 4337	1203.25 (34.55)
36.	BH 4371	1003.0 (31.65)
37.	K 7792	830.0 (28.69)
38.	K 7793	1340.0 (36.53)
39.	C 306	1382.25 (37.10)
40.	C 518	515.75 (22.68)
41.	DL 39-5	375.75 (19.19)
42.	SL 79-1	955.0 (30.89)
43.	IWP-72	470.5 (21.6)
44.	HP 1345	634.5 (25.02)
45.	HD 2280	876.0 (29.39)
46.	HS-81	730.5 (27.23)
	CD : P=0.05	3.57
	P=0.01	4.72

Figures in parentheses are mean of \sqrt{n} transformation, the figures outside the parentheses are the original mean values.

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REPORTS AND NEW RECORDS

REPORT OF *LEPTOCENTRUS TAURUS* FABRICIUS (MEMBRACIDAE : HOMOPTERA) FEEDING ON *PARTHENIUM HYSTEROPHORUS* LINN.

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The congress grass, *Parthenium hysterophorus* LINN. is a noxious weed, establishing and colonising very easily in the cultivated fields and fallow lands in many parts of India. It poses serious problem to our agriculture and health (KRISHNAMOORTHY *et al.*, 1975). It is known to be resistant to pests and diseases. However, SUNDARA RAJULU *et al.* (1976) reported *Aphis fabae* feeding on *Parthenium* at Coimbatore, and suggested the possible use of this aphid in the biological control of this dangerous weed. *A. fabae* is distributed only in the temperate regions and so far not reported in tropical India, hence its record on *Parthenium* at Coimbatore needs further confirmation and during this survey also *A. fabae* could not be recorded.

During a survey for insects feeding on the weeds, the cowbug *Leptocentrus taurus* Fabr. was found feeding on *Parthenium* at Mysore (opposite Doddakere maidan) during Sept.—Oct., 1976. Subsequently this cowbug infestation on *Parthenium* was noticed in the different localities of Mysore, particularly during August–November in the year 1979. The adults were invariably found scattered on the main stem in between the nodes while the nymphs were in small colonies; five to seventeen nymphs were found in the various colonies. Nymphs were noticed mainly on the petioles and

base mid-ribs on the under surface of leaves, which afford suitable sites for the feeding of the nymphs. Slightly over a thousand plants were individually examined for the presence of the cowbug. About 20% of the plants were infested by either adult or nymphs or both and such plants were easily recognised by the presence of the common larger black ant, *Camponotus compressus* which attends on the adults and nymphs of *L. taurus* for the “honey-dew”.

The adults of this bug occasionally feed on cotton also and this is the first record of this cowbug feeding on *Parthenium* and cotton. Other than *L. taurus* stray incidence of the nymphs of the spittle bug (Cercopidae: Homoptera), adults of *Chrysocoris* sp. (Scutelleridae: Heteroptera) were also noticed on *Parthenium* at Mysore during this investigation.

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A NEW HOST FOR THE ENTOMOGENOUS FUNGUS *PENICILLIUM OXALICUM* CURRIE AND THOM

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While making a survey of entomogenous fungi associated with crop pests in Kerala

during June–July 1978, the authors came across an epizootic infection of *Cicadella spectra* at Vellayani.

The dead hoppers were collected from the field and a fungal pathogen was isolated in pure culture on potato dextrose agar. It was identified as *Penicillium oxalicum* CURRIE & THOM and artificial inoculation of the fungus to healthy hoppers by spraying spores from 5 day old cultures revealed that it was highly pathogenic causing more than 90 per cent mortality within 3 days.

The infected rice leafhoppers exhibited a gradual decrease in activity and reaction to touch. Colour changed to light green as against the white colour of healthy ones. Just before death there was general softening of the body and later the cadavers became stiff and mummified. The dead hoppers were found anchored firmly on leaf surface with their wings stretched. External myce-

lium appeared within 48 to 72 hours of death. The fungal colonies on artificial cultures were broadly spreading, heavily sporulated and dark blue in colour.

DELFINADO (1959) recorded the incidence of *Penicillium* sp. on the larvae of *Tryporyza incertulas* in Philippines. In India incidence of mortality of *Cicadella spectra* by *Syncephalastrum racemosum* has been reported earlier by MATHAI *et al.* (1979).

Acknowledgements:—Thanks are due to Commonwealth Mycological Institute Kew Surrey, London for identifying the fungus.

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